

Figure 1

302720 - 22288860

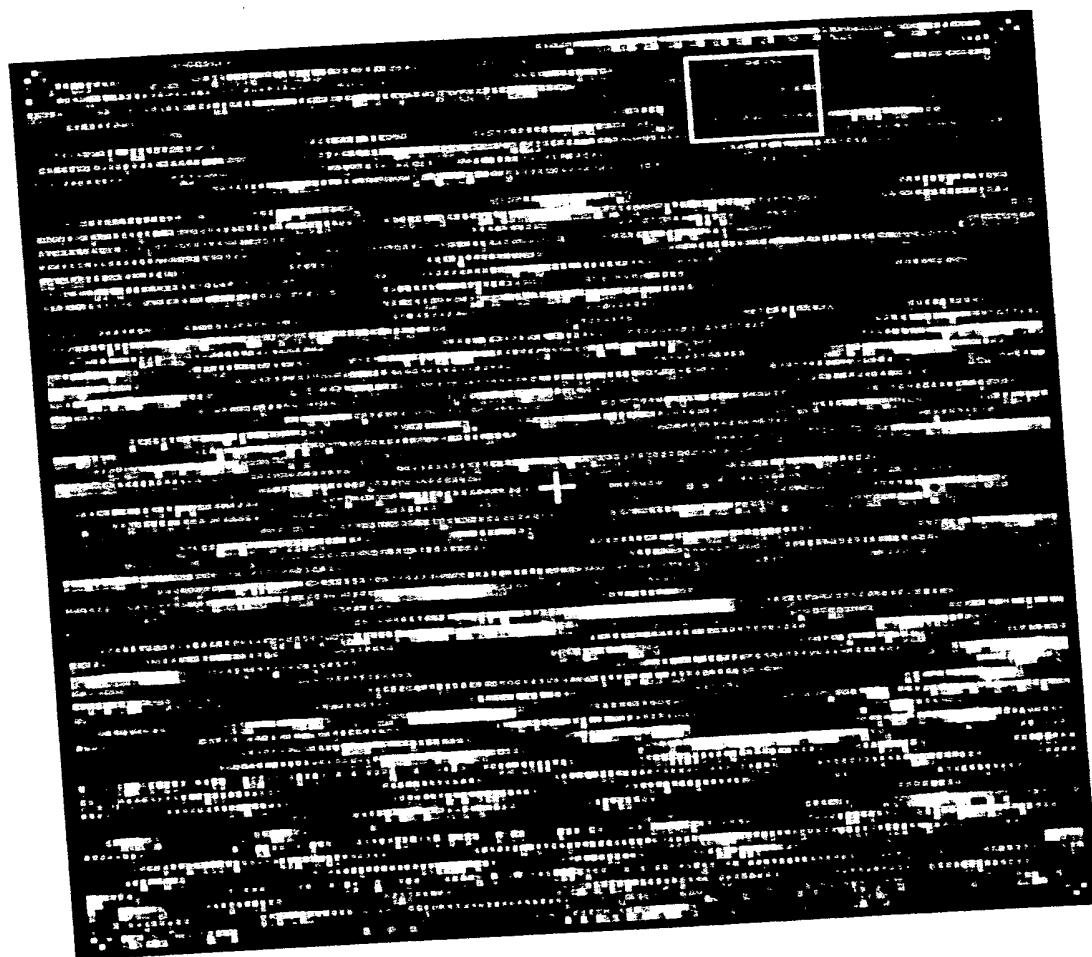


Figure 2a

Figure 2b

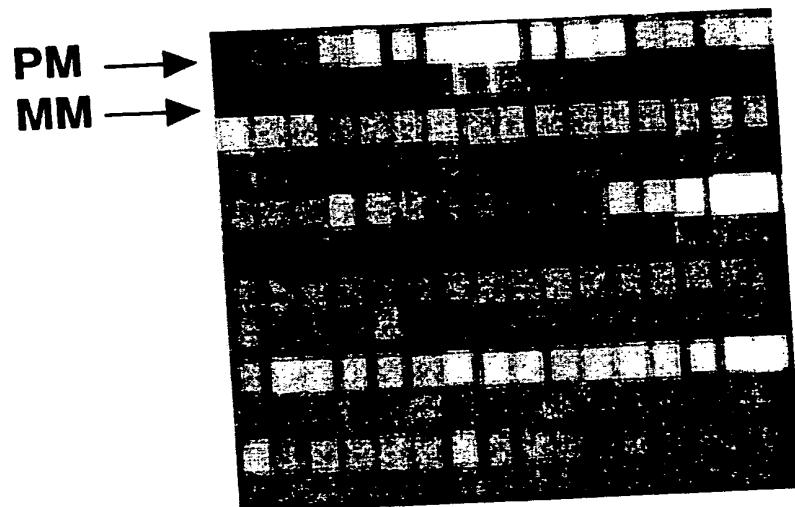
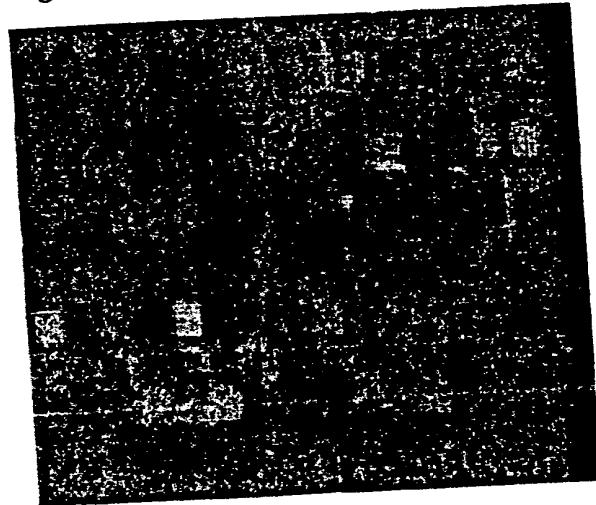


Figure 2c



4337300-222000000

Hybridization Signal vs Target Concentration

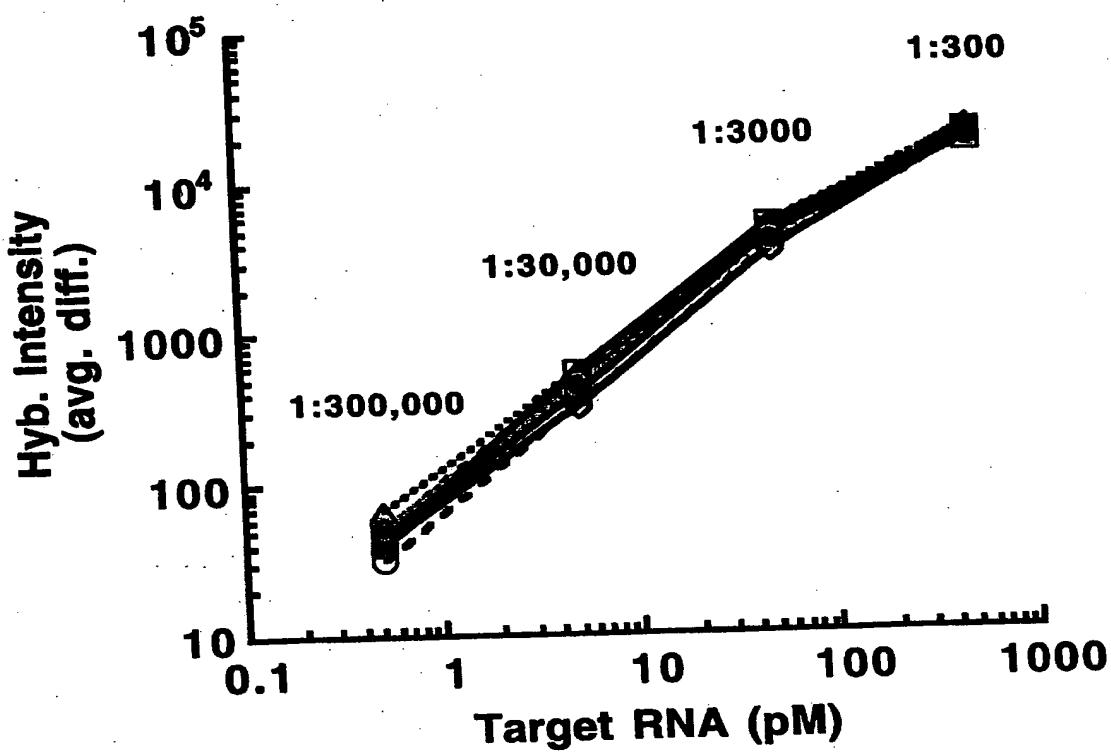


Figure 3

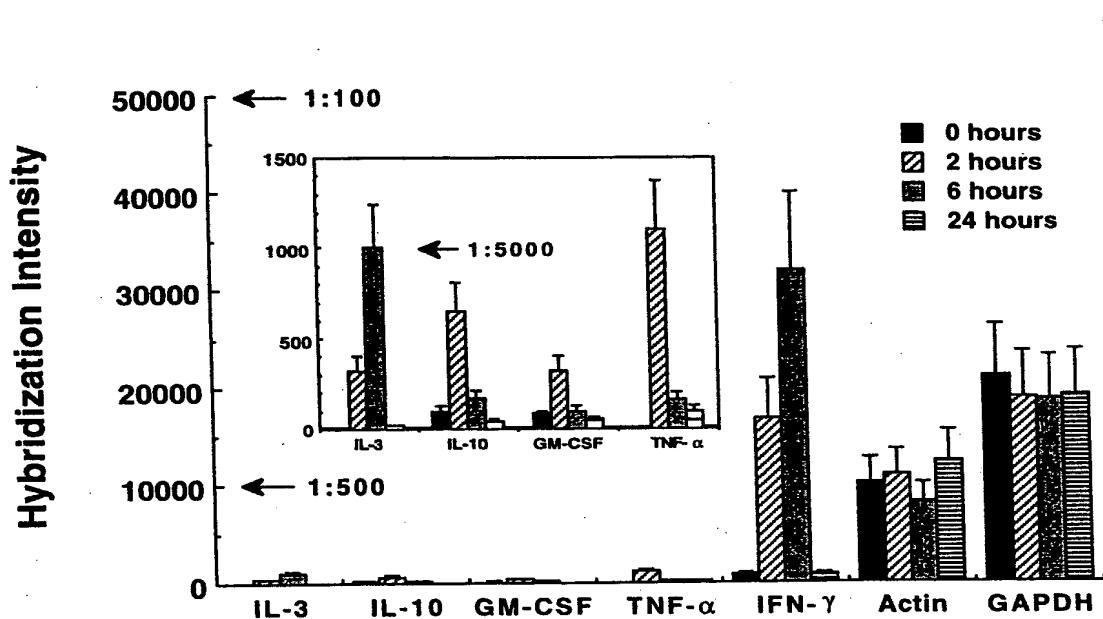


Figure 4

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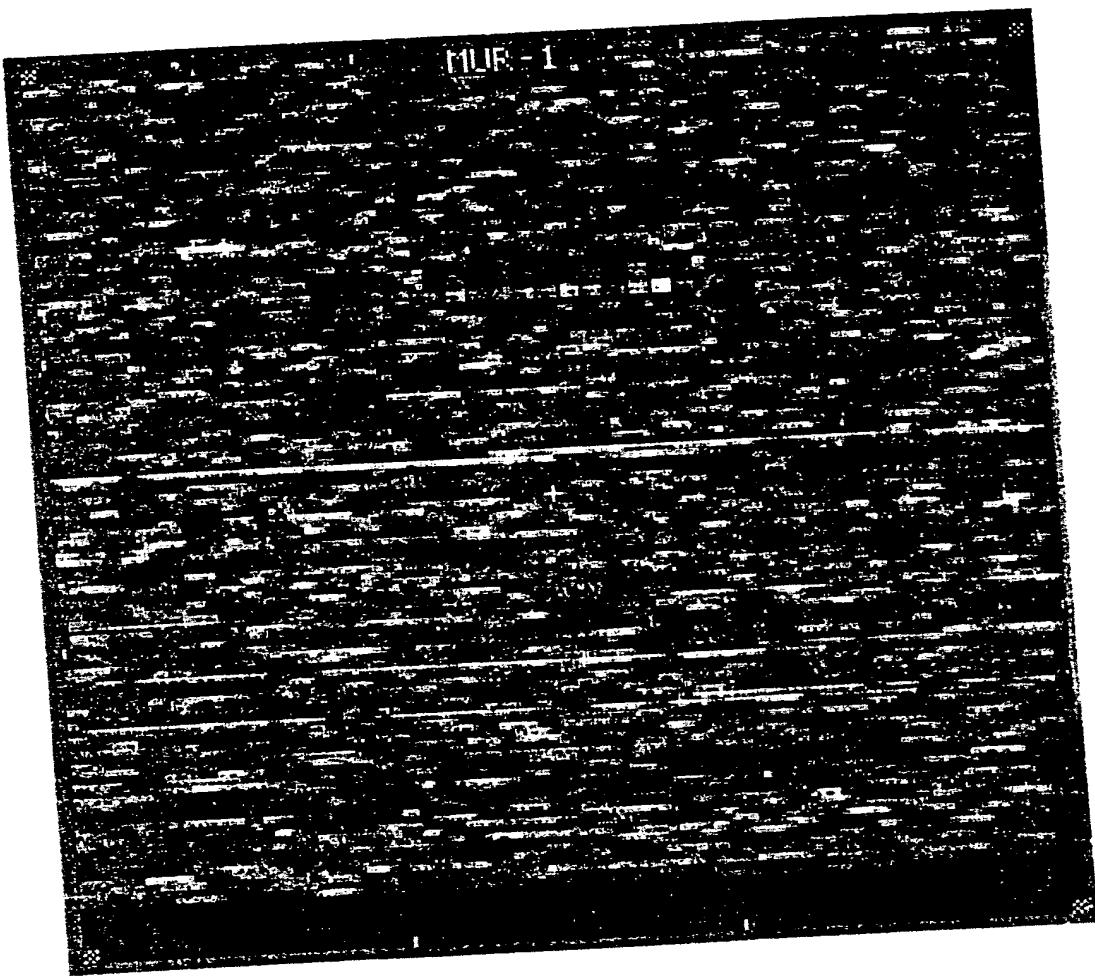


Figure 5

032307227 • 06642014

9 32 3 30 2 22 22 26 8

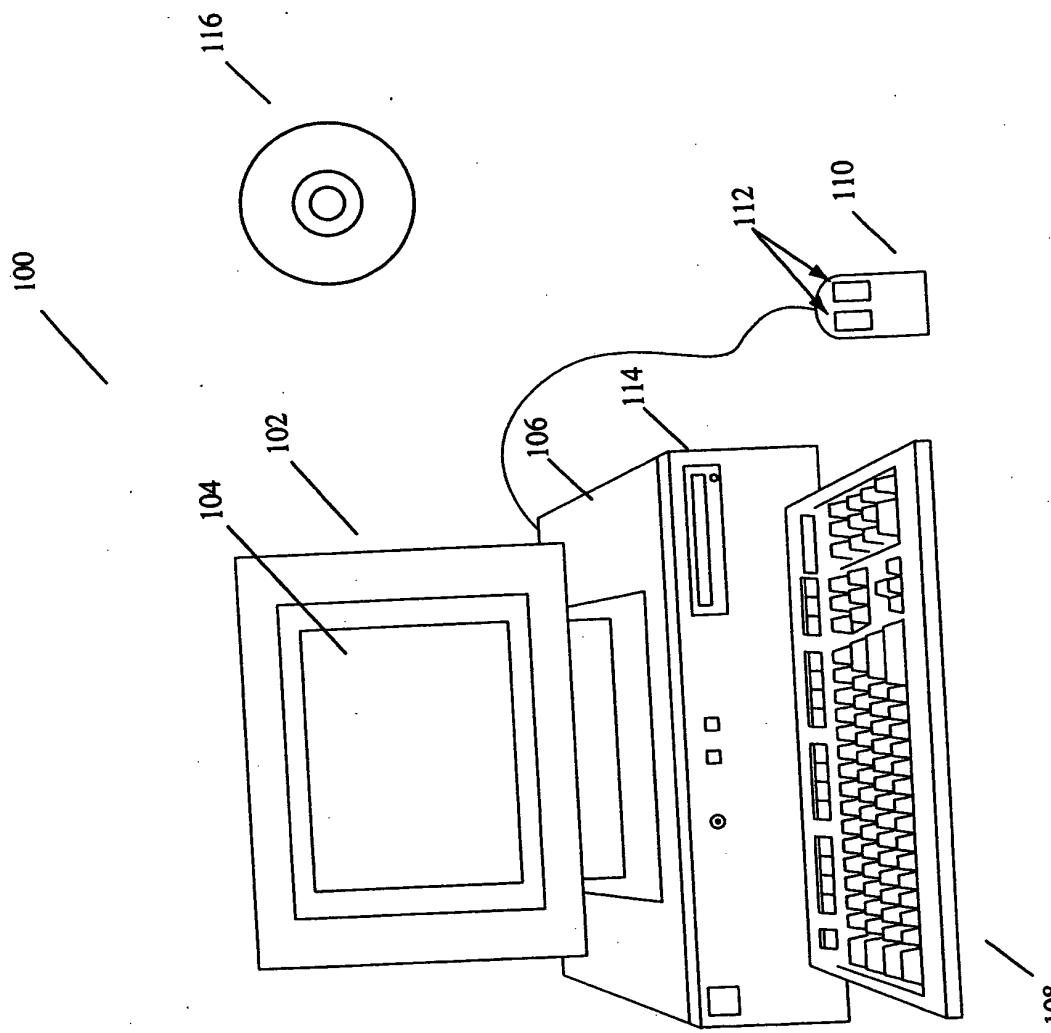


Figure 6

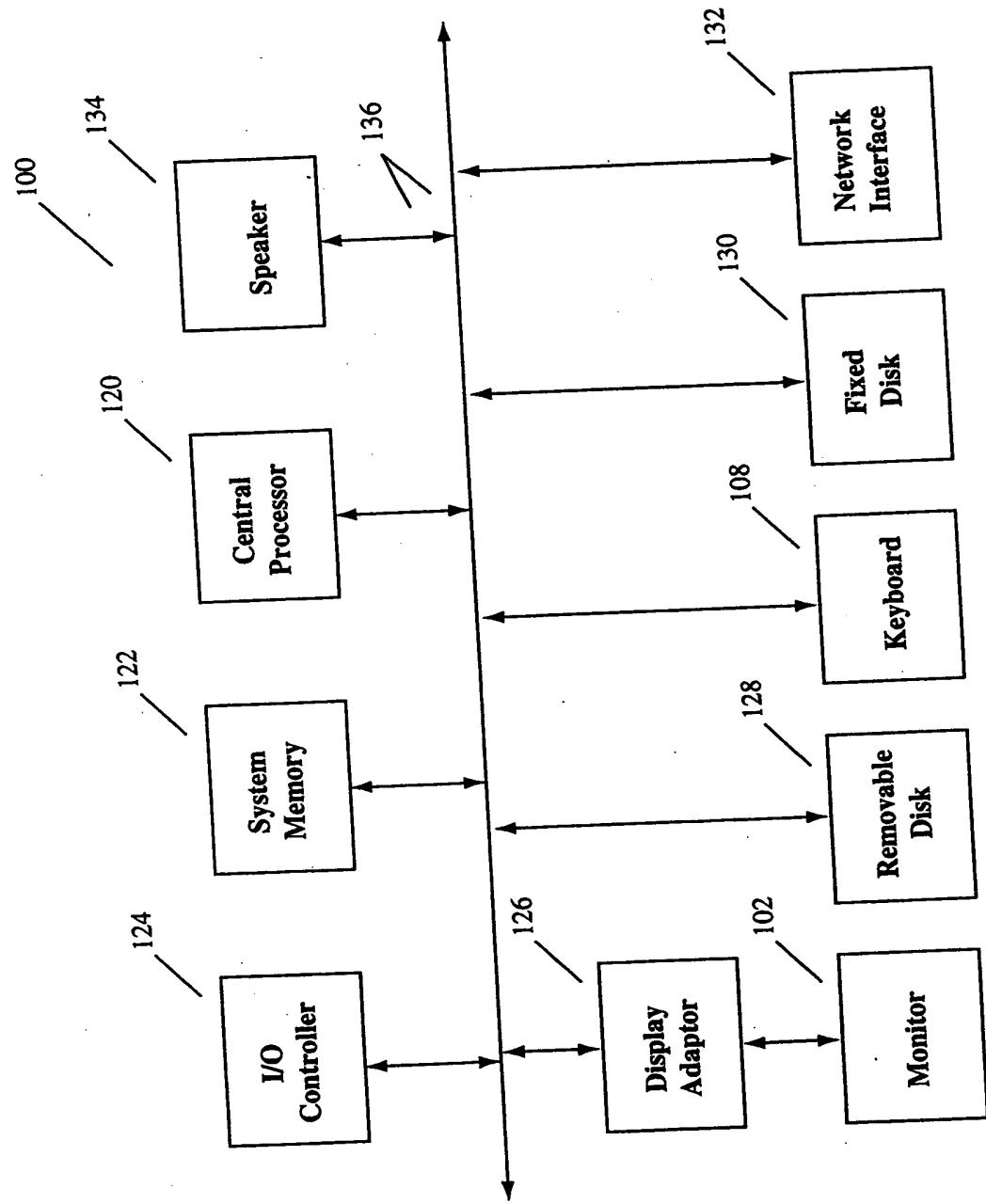


Figure 7

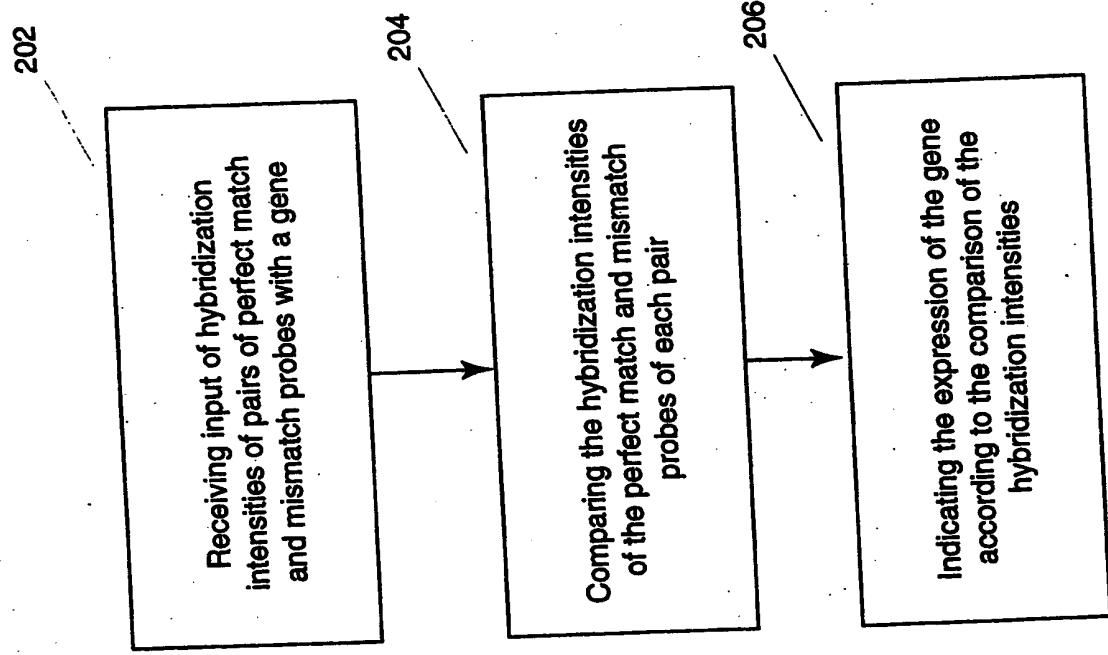


Figure 8

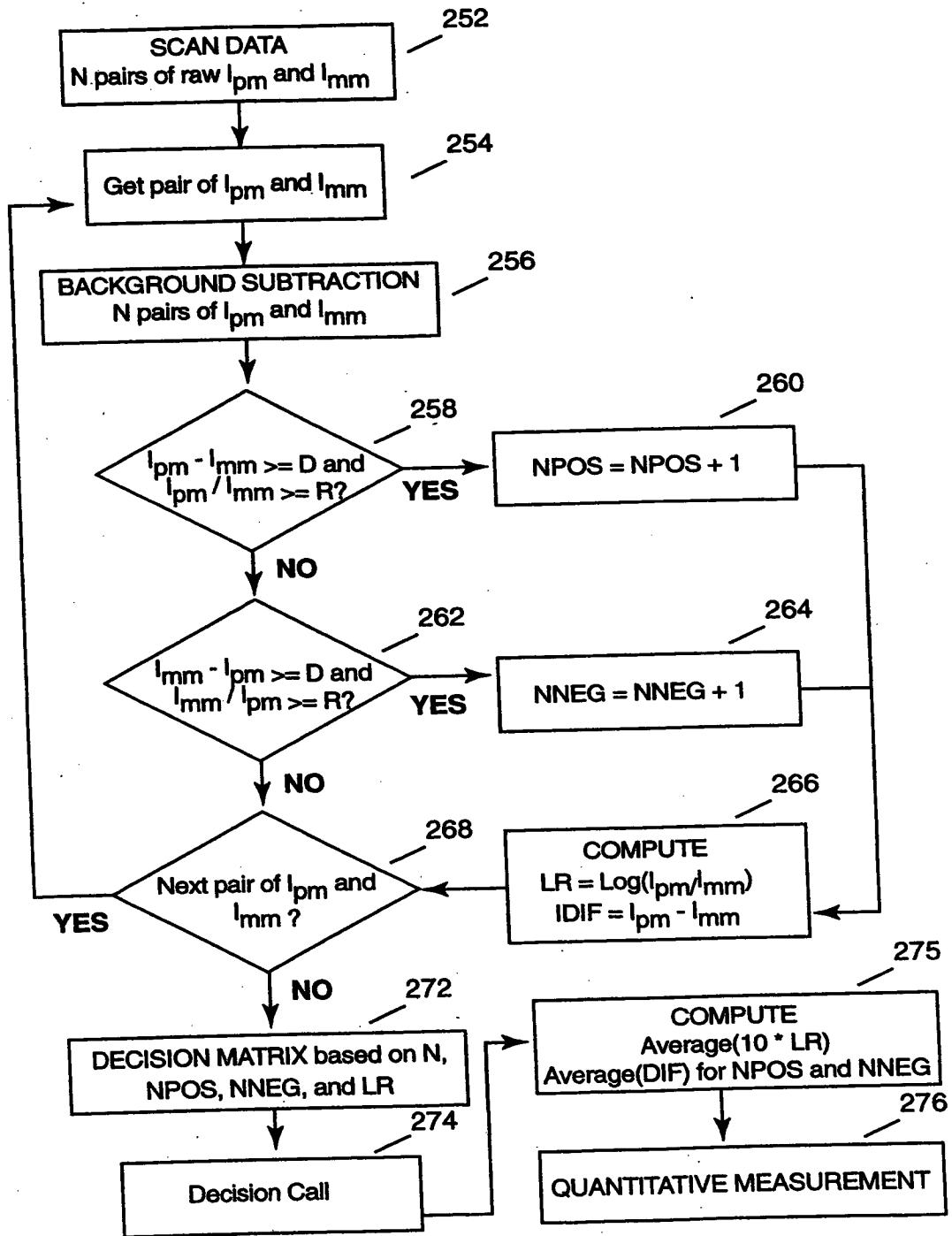


Figure 9

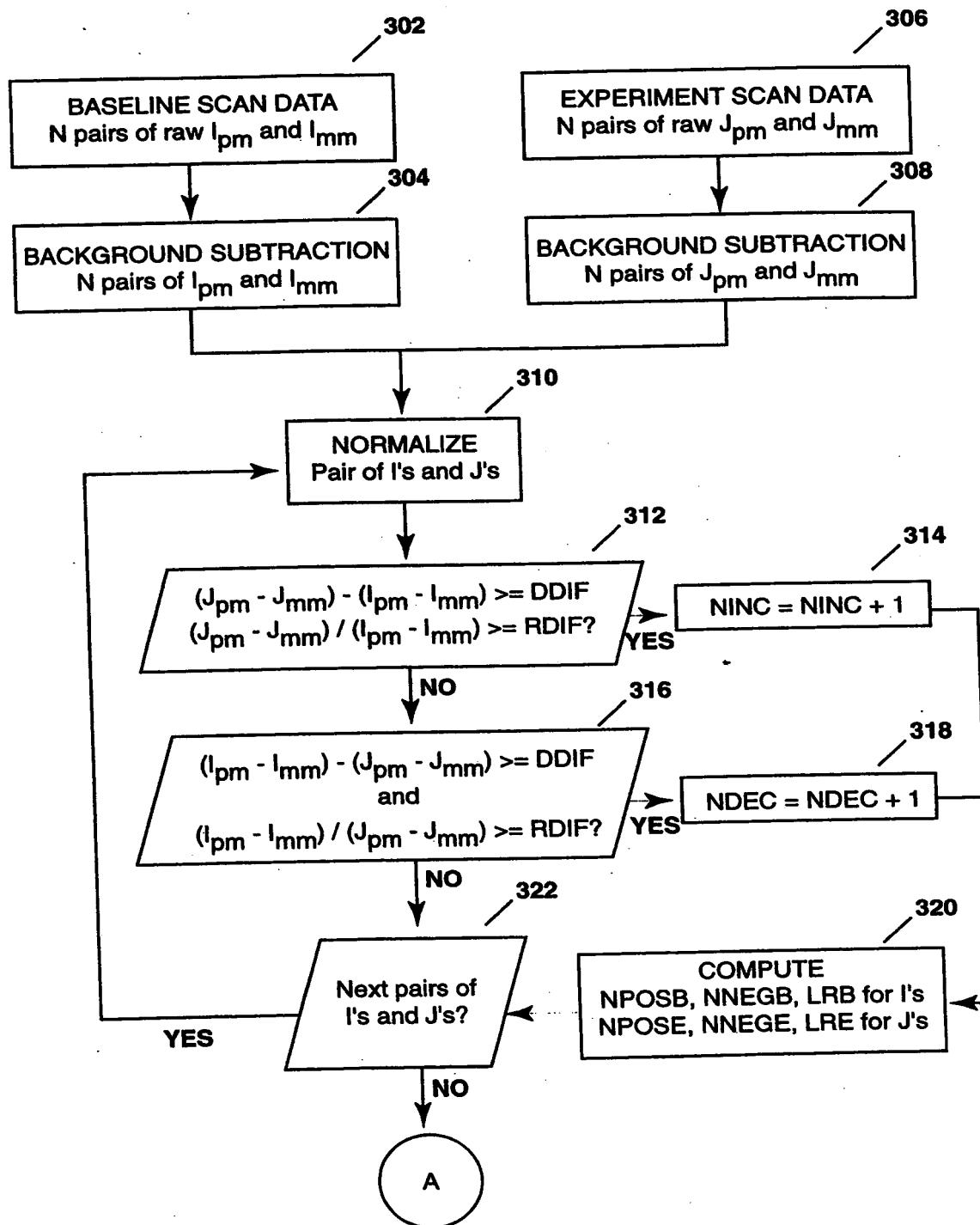


Figure 10a

12/47

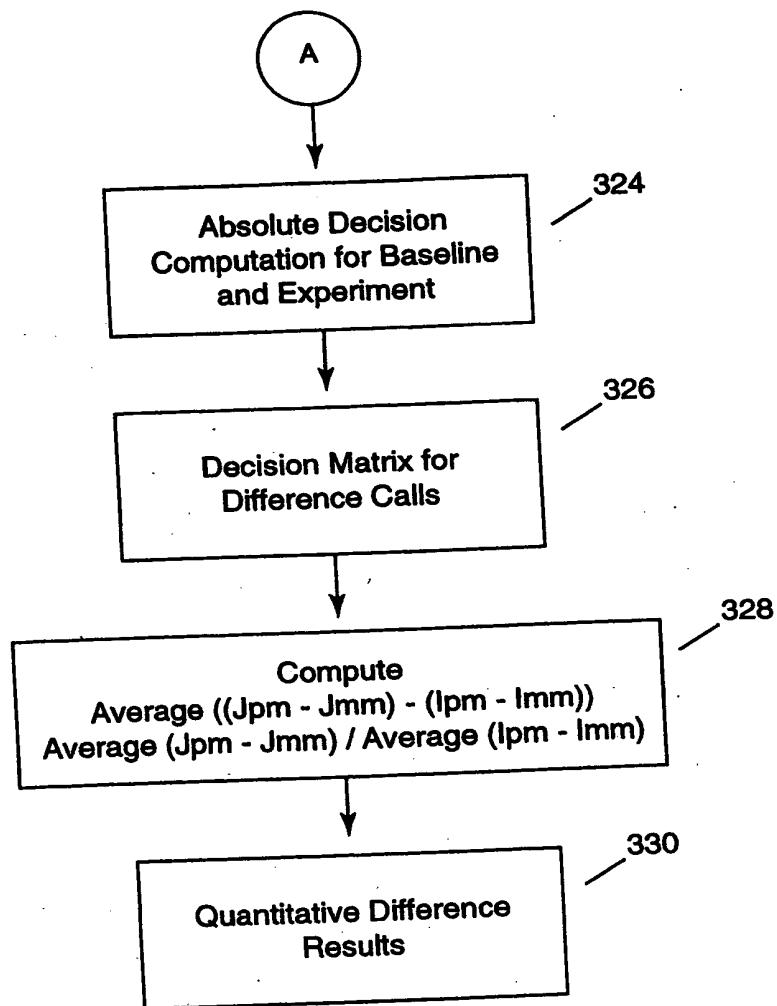


Figure 10b

402 404 406 410 412 414 416 418 420 422 424 426 428 430 432 434

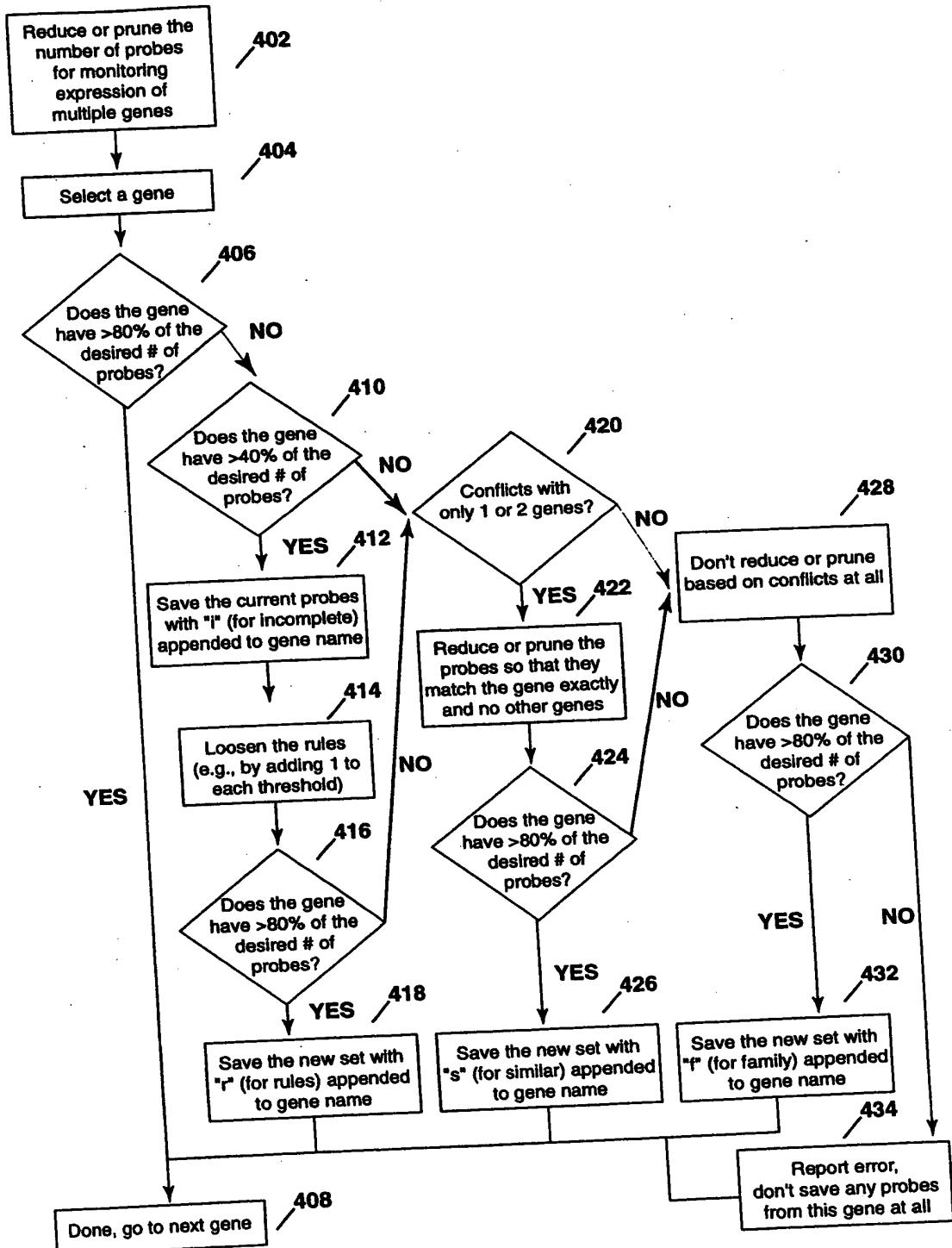
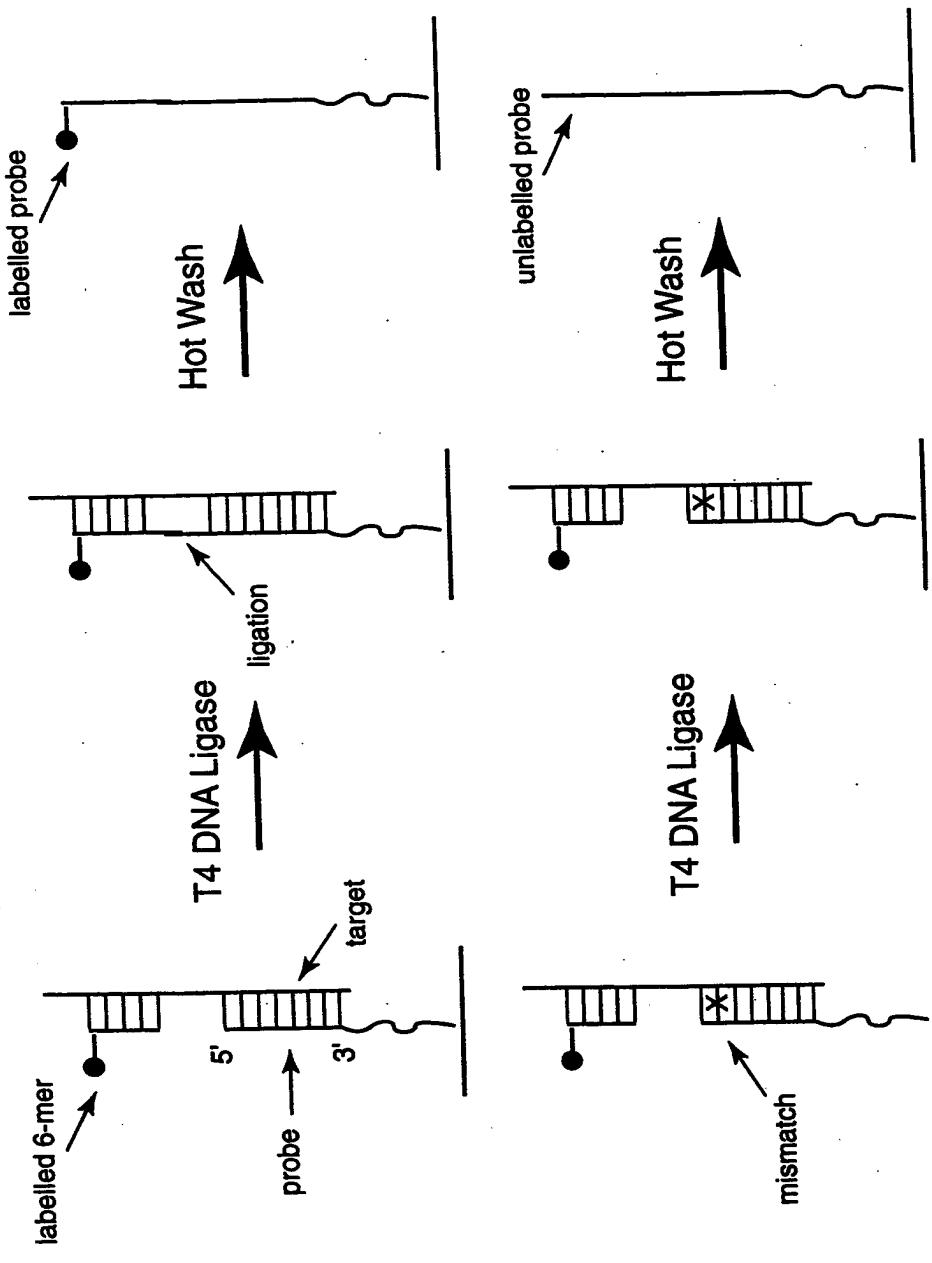


Figure 11

Discrimination with Ligation



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Figure 12

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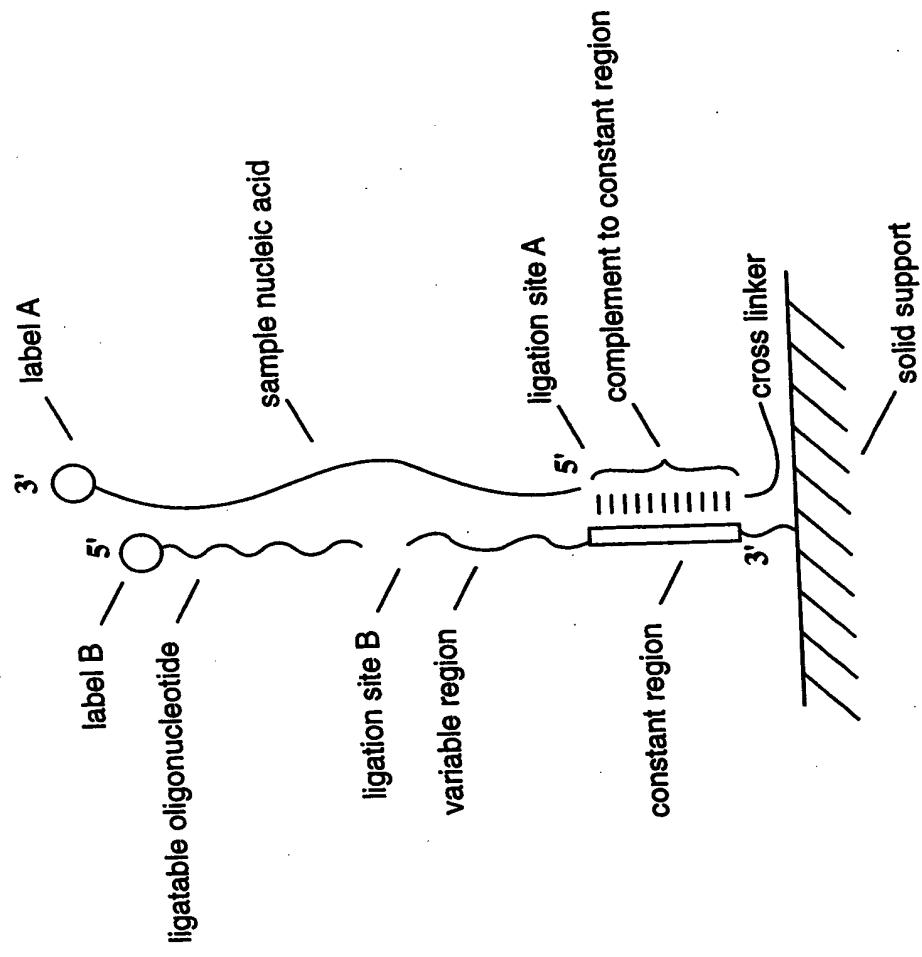


Figure 13a

Figure 13b

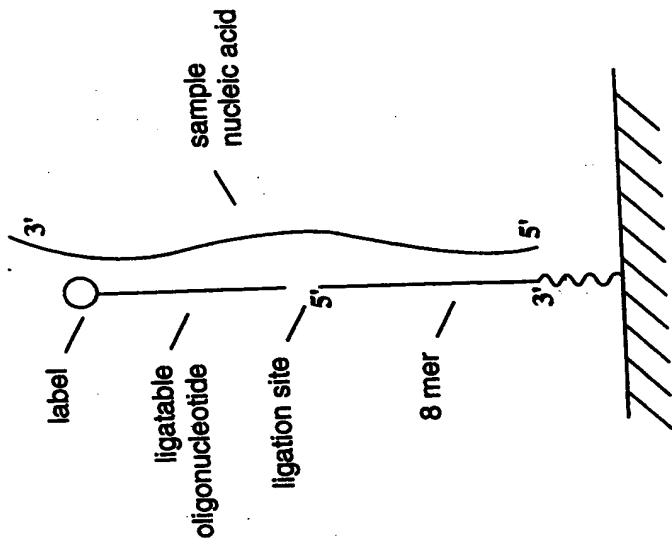
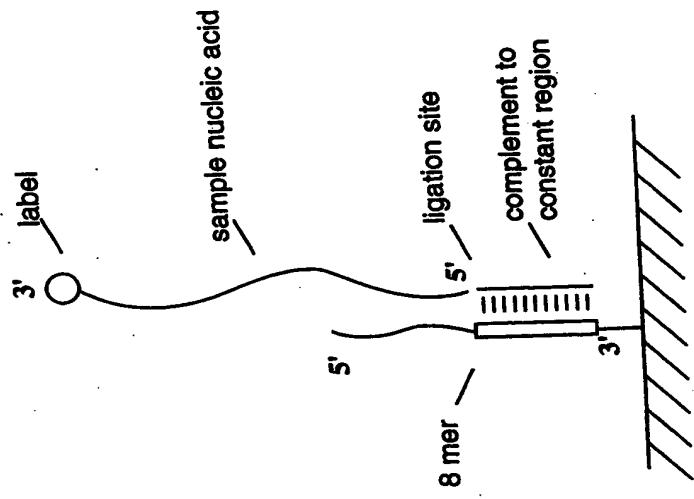


Figure 13c



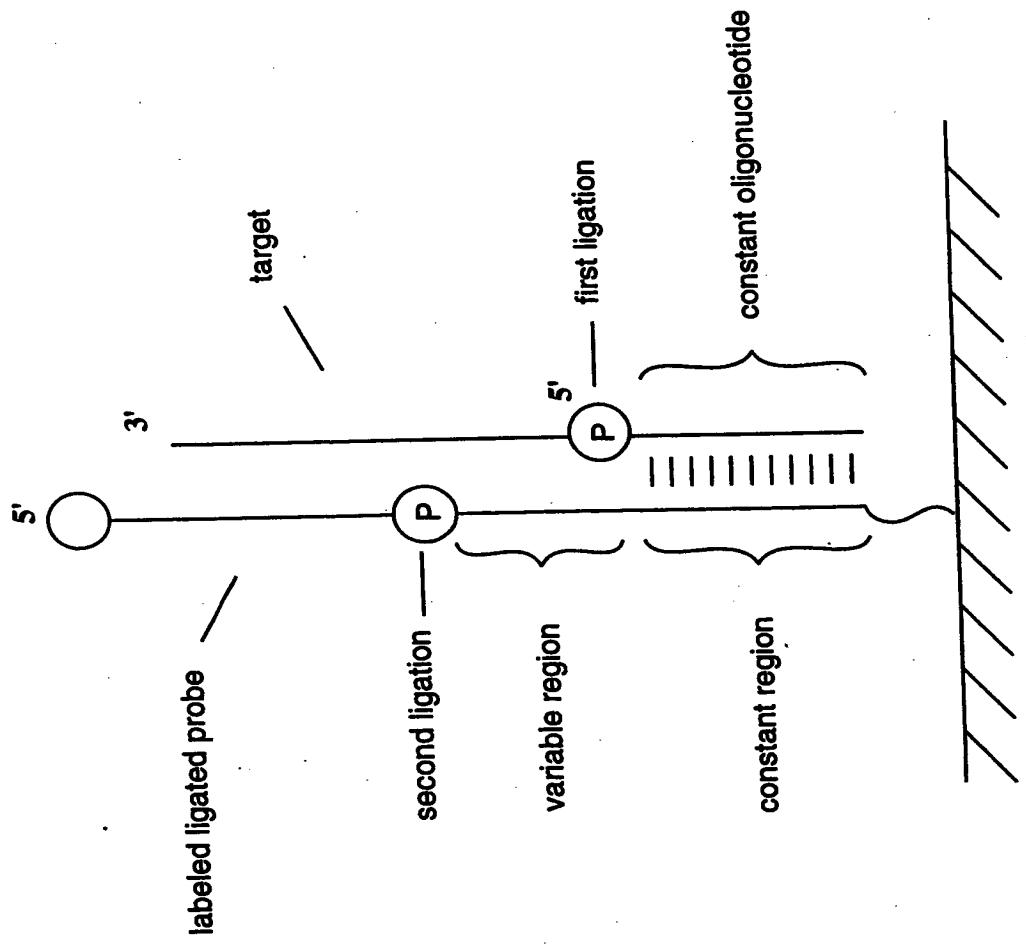


Figure 13d

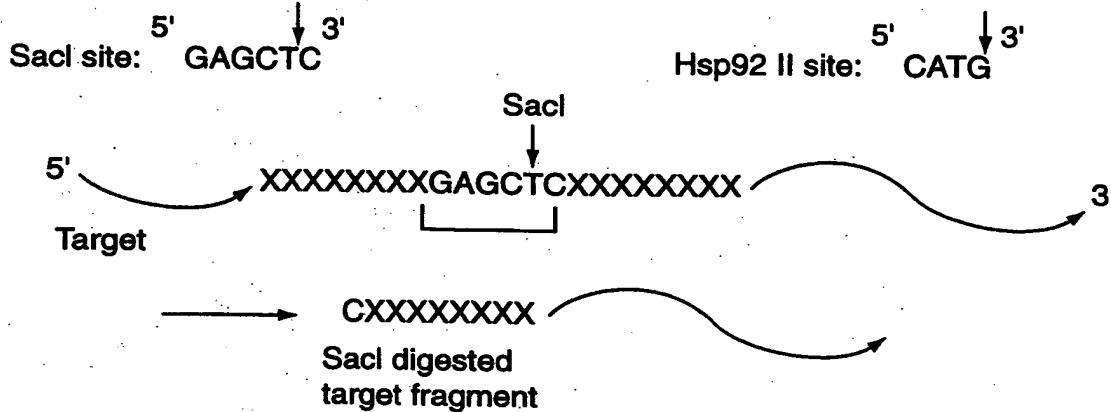
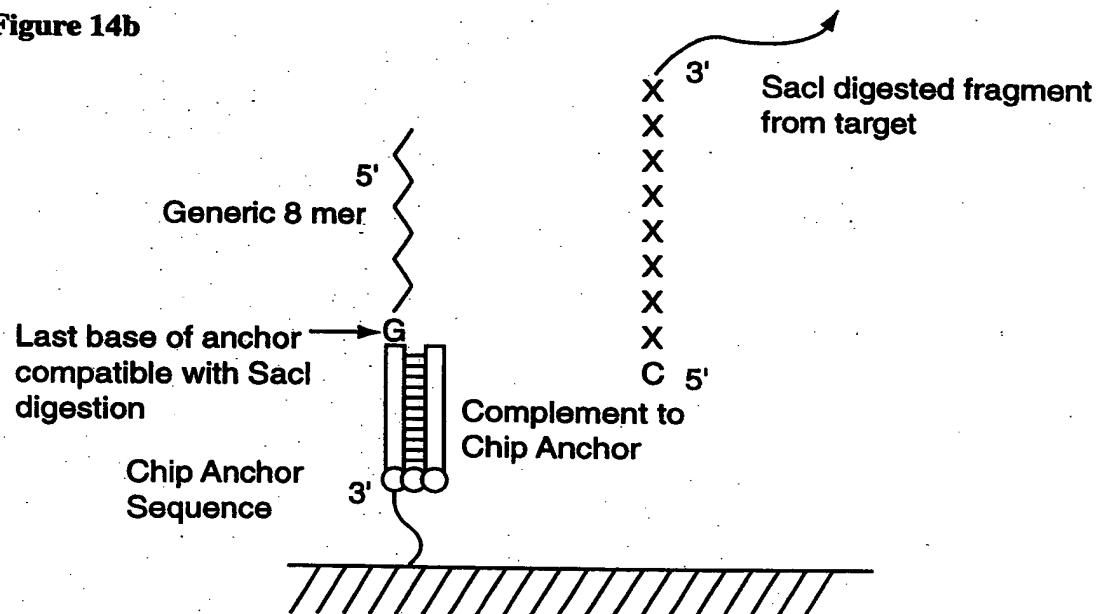
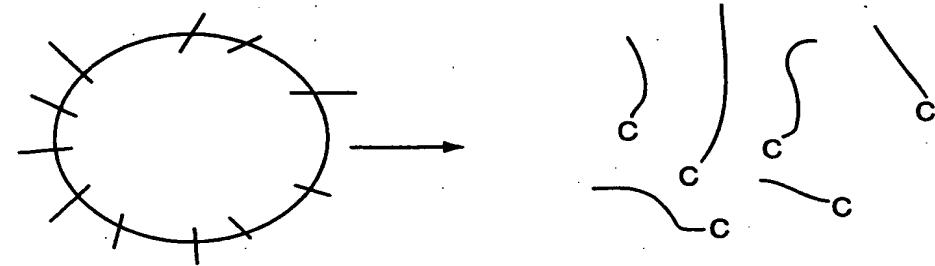
Figure 14a**Figure 14b**

Figure 14c

Monitoring mRNA expression from organisms with small genomes:



6 Mb genome or cDNA library

~ 1 kb genomic or cDNA
fragments with 5' C

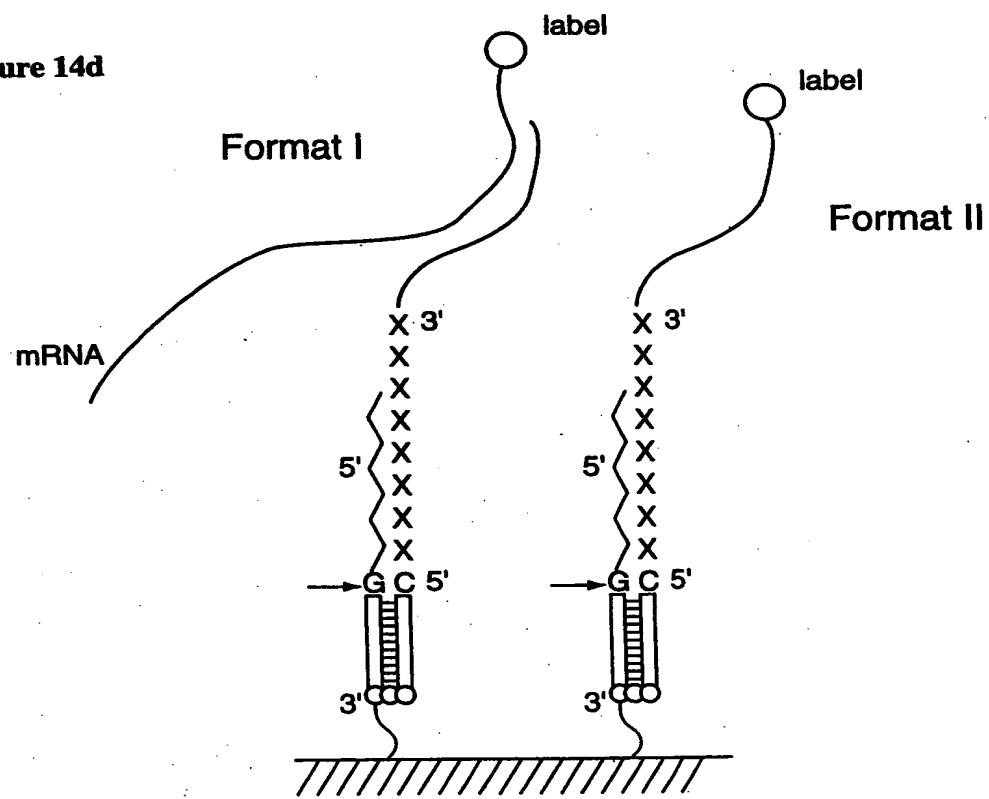
Figure 14d

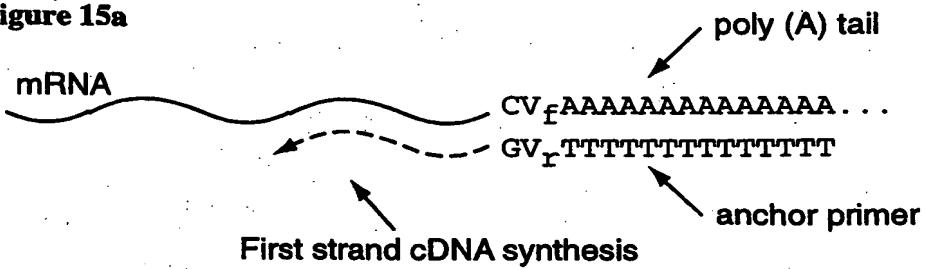
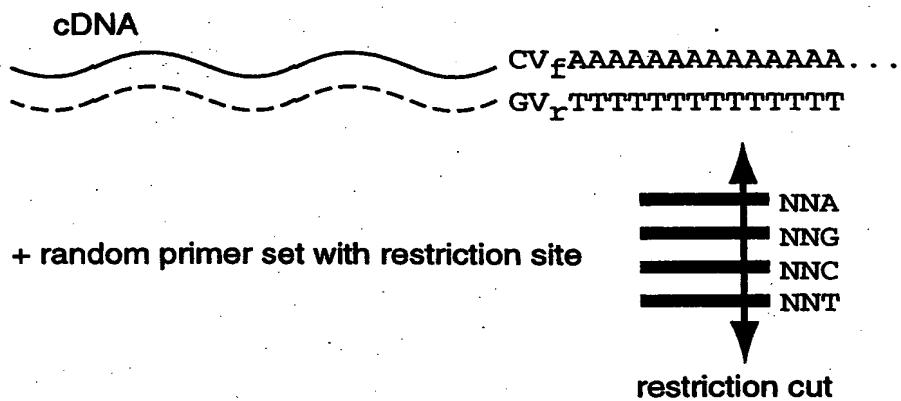
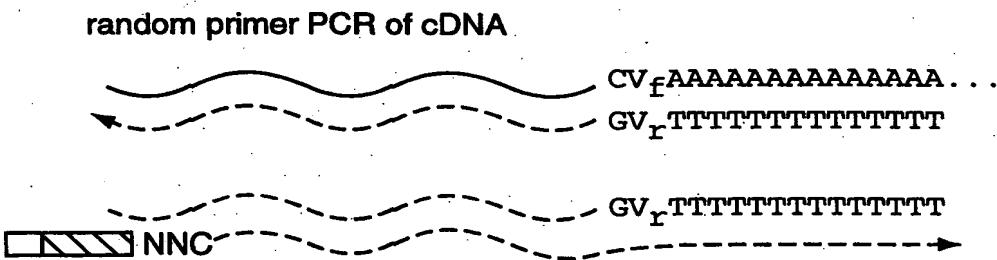
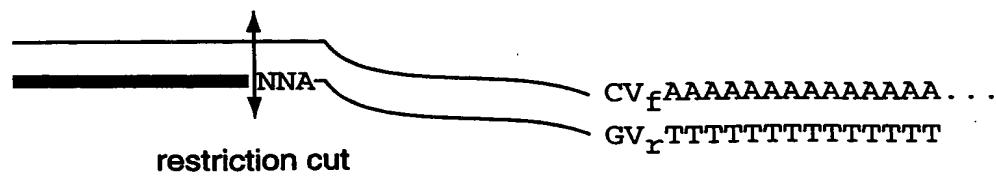
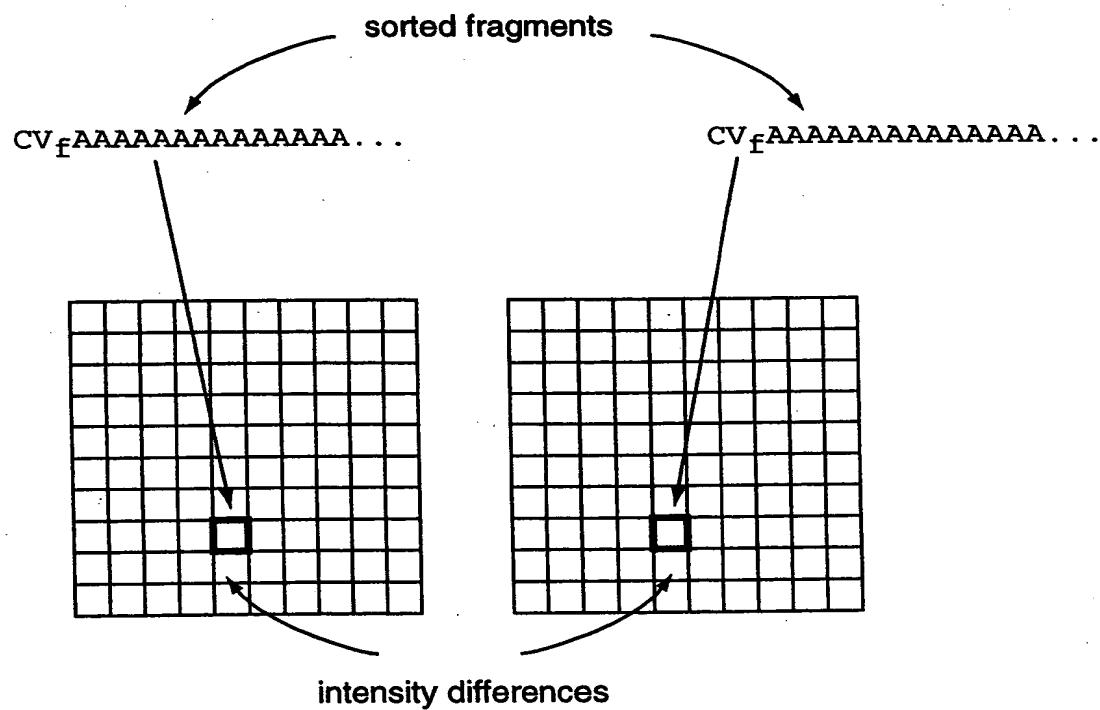
Figure 15a**Figure 15b****Figure 15c**

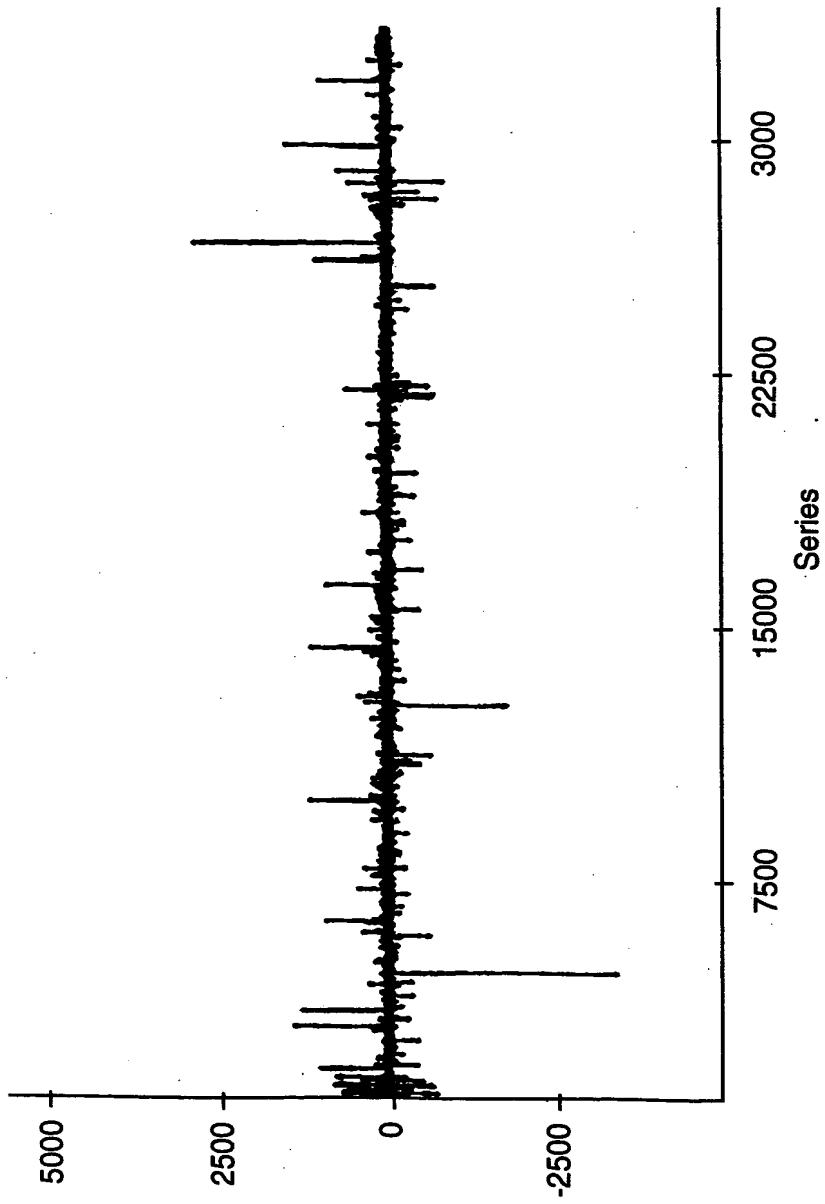
Figure 15d

Restriction digest PCR products

**Figure 15e**

Sort fragments by 5' ends on Generic Ligation GeneChip



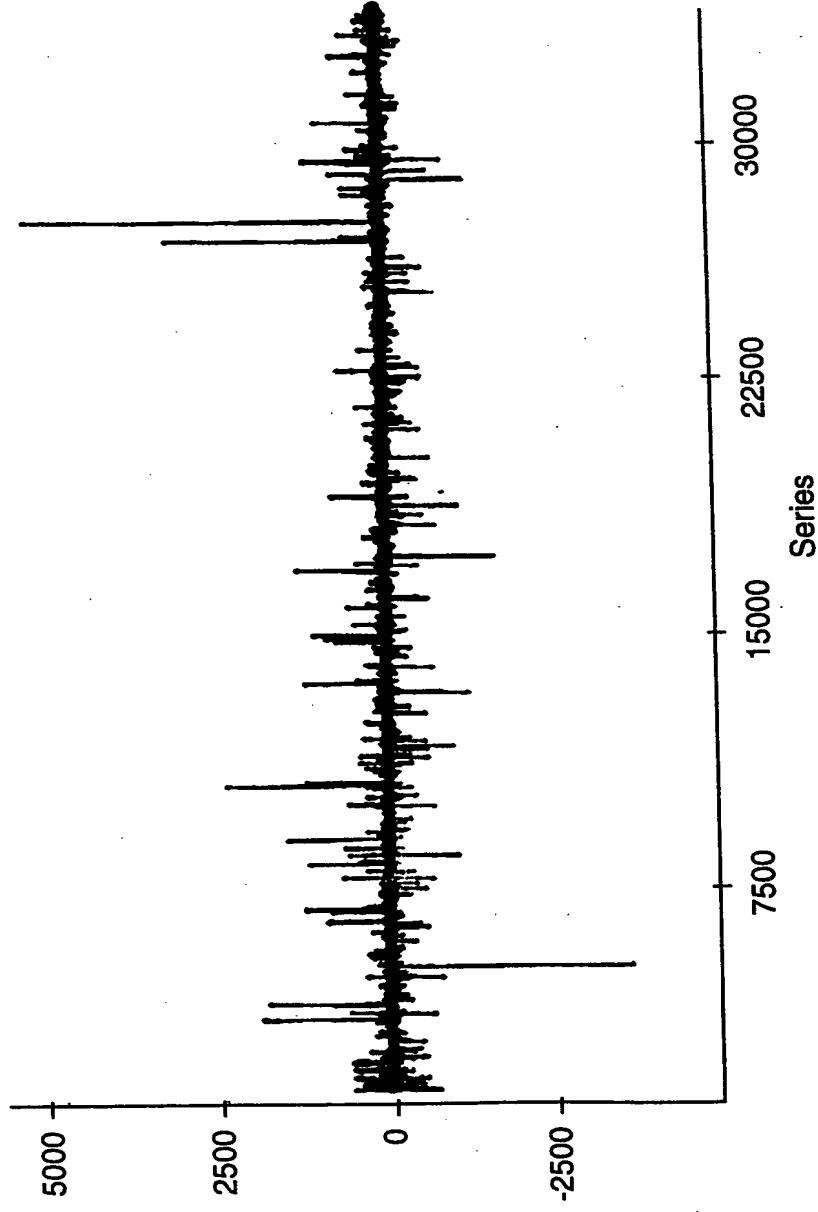


**Sample 1 vs. Sample 1 - Absolute Differences
(Replicate 1 vs. Replicate 2)**

Figure 16a

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Sample 2 vs. Sample 2 - Absolute Differences
(Replicate 1 vs. Replicate 2)

Figure 16b

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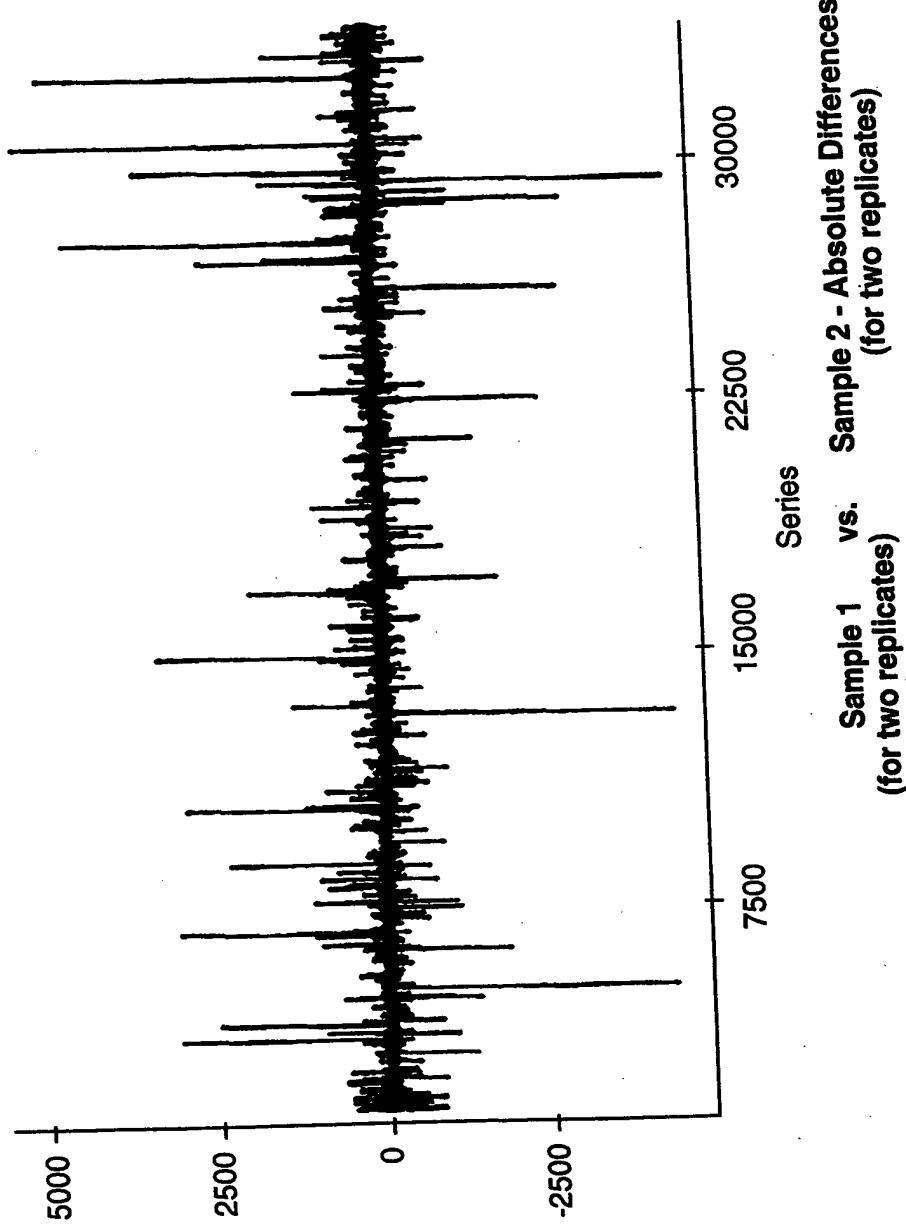


Figure 16c

25/47

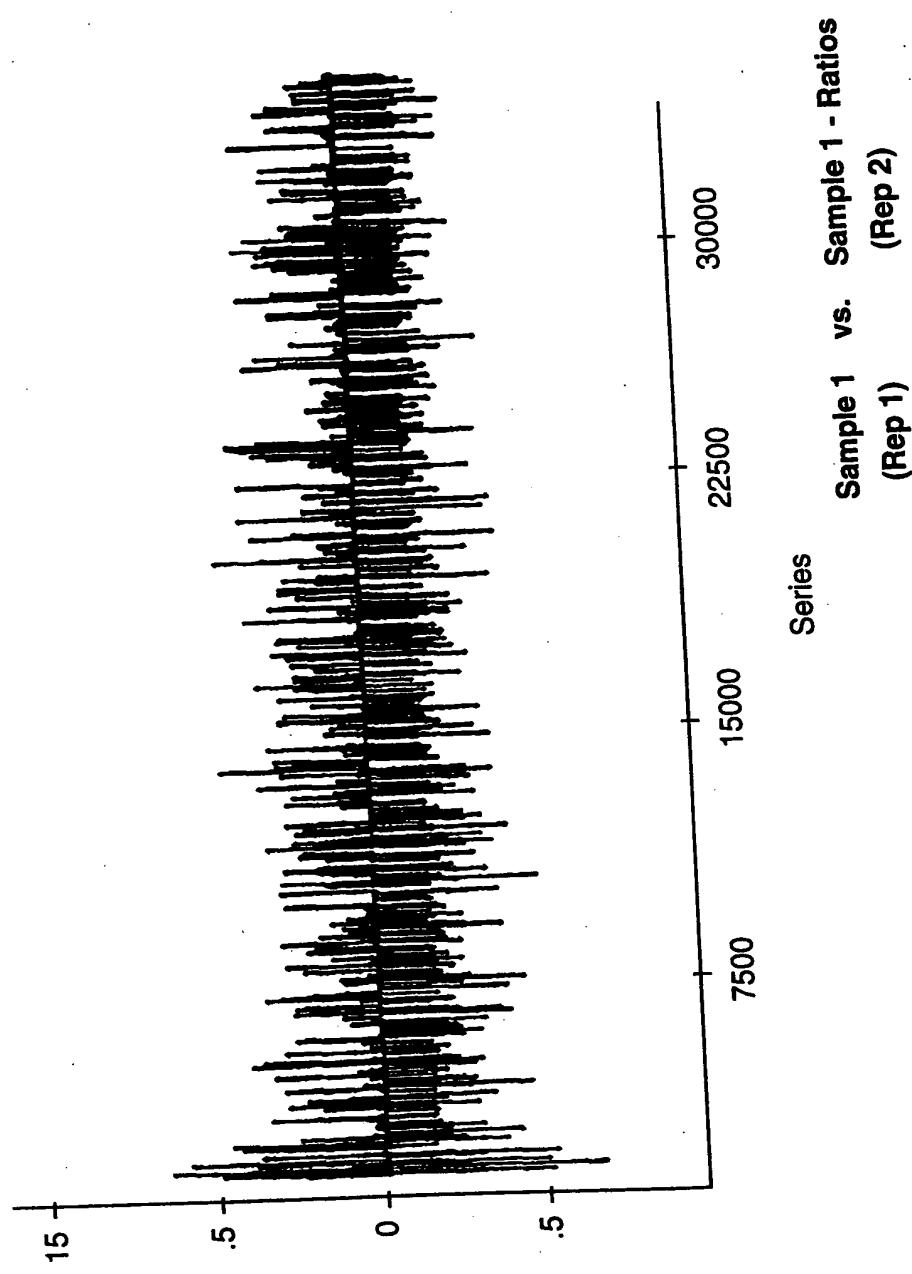


Figure 17a

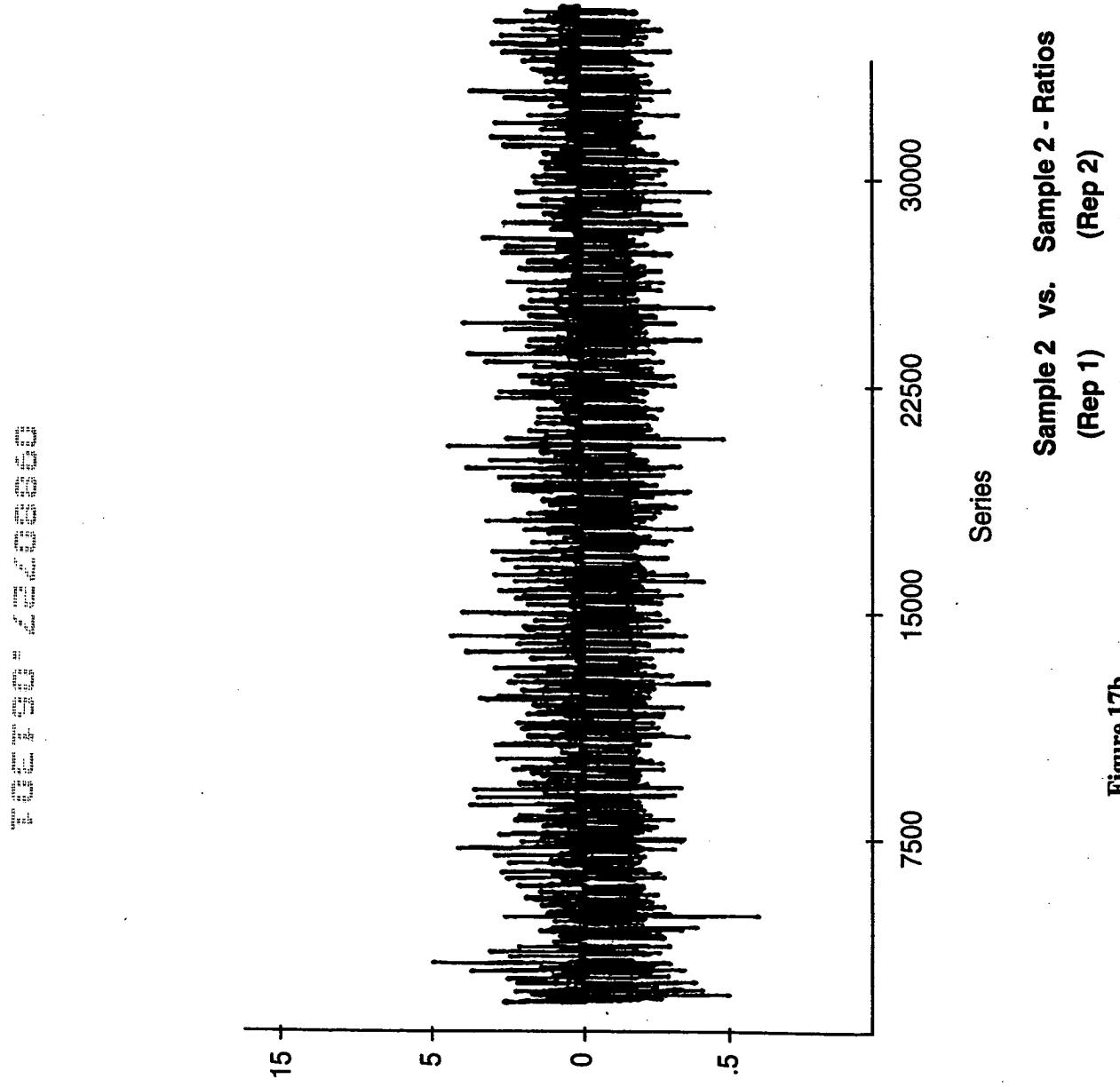


Figure 17b

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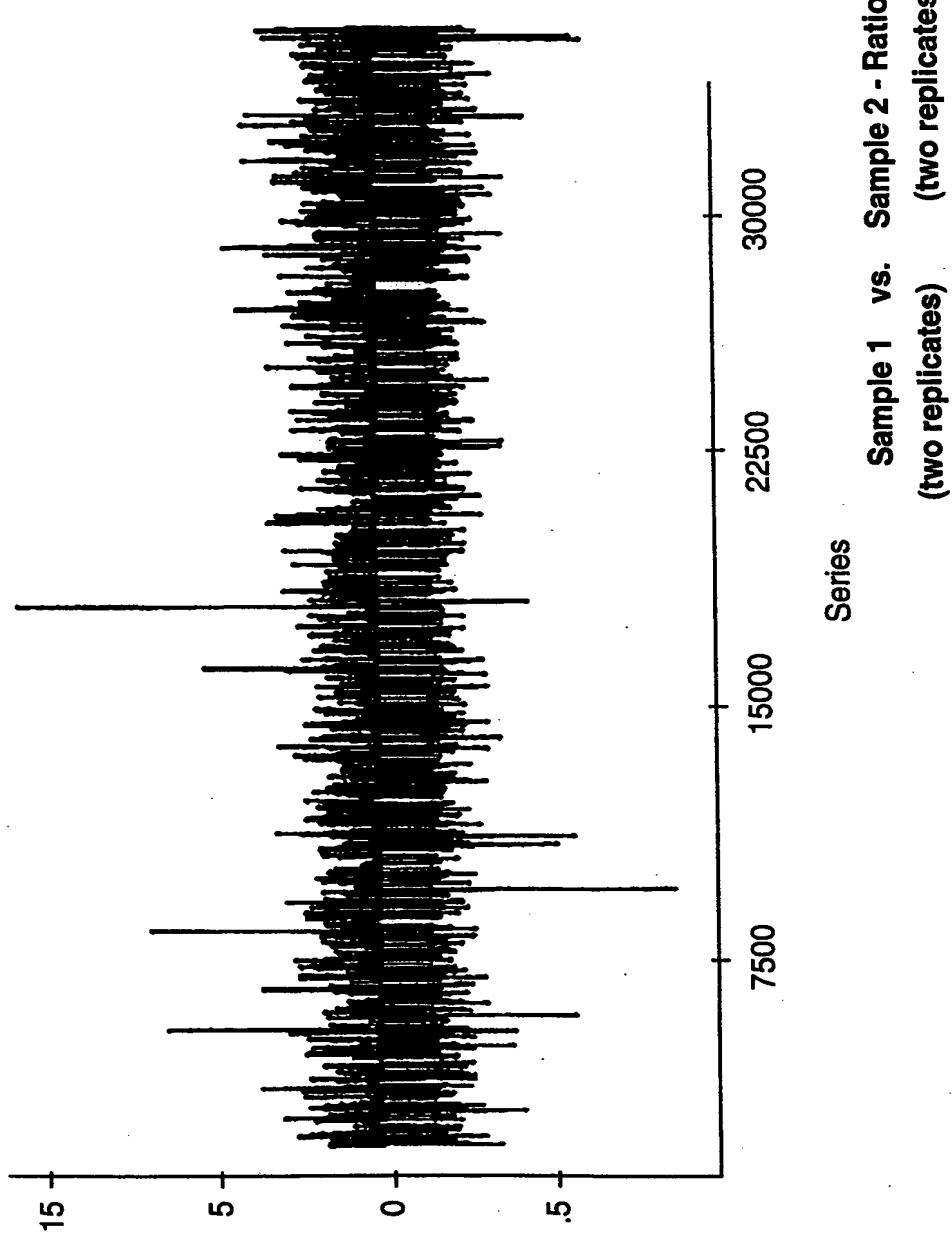
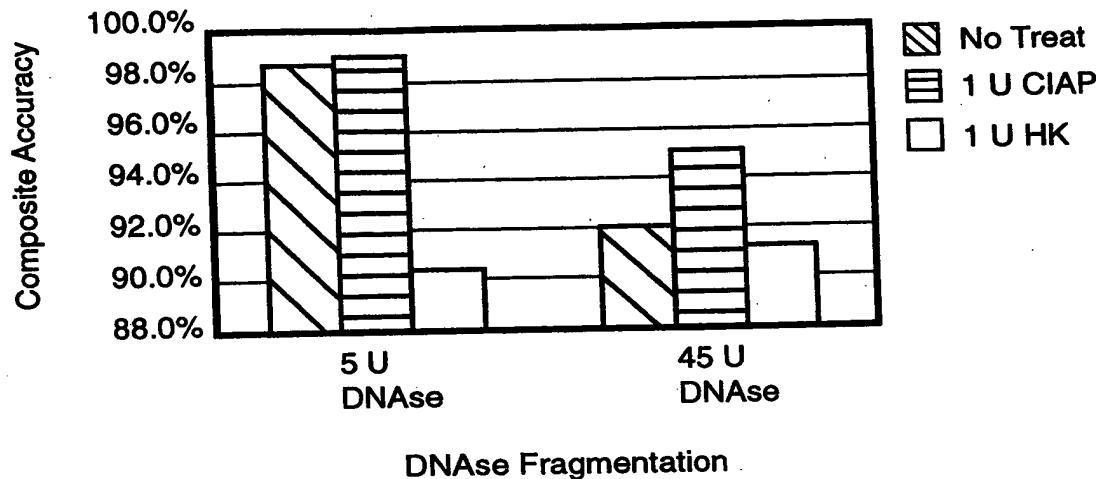


Figure 17c

Post-Fragmentation End Labeling: CIAP Treatment

25 U TdTase: 1 nmol FITC-ddUTP



25 U TdTase: 1 nmol FITC-ddUTP

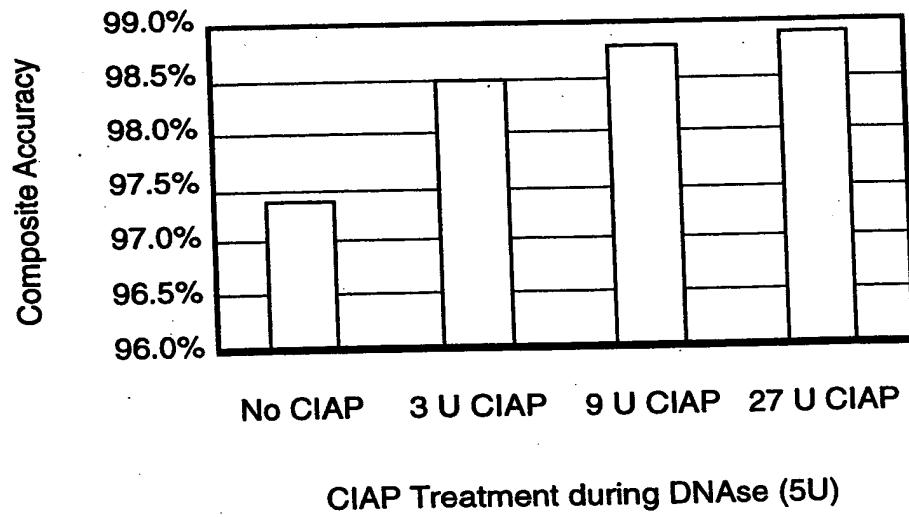


Figure 18

Post-Hybridization End Labeling on the Chip

F 3' OH 5' OH OH Probe

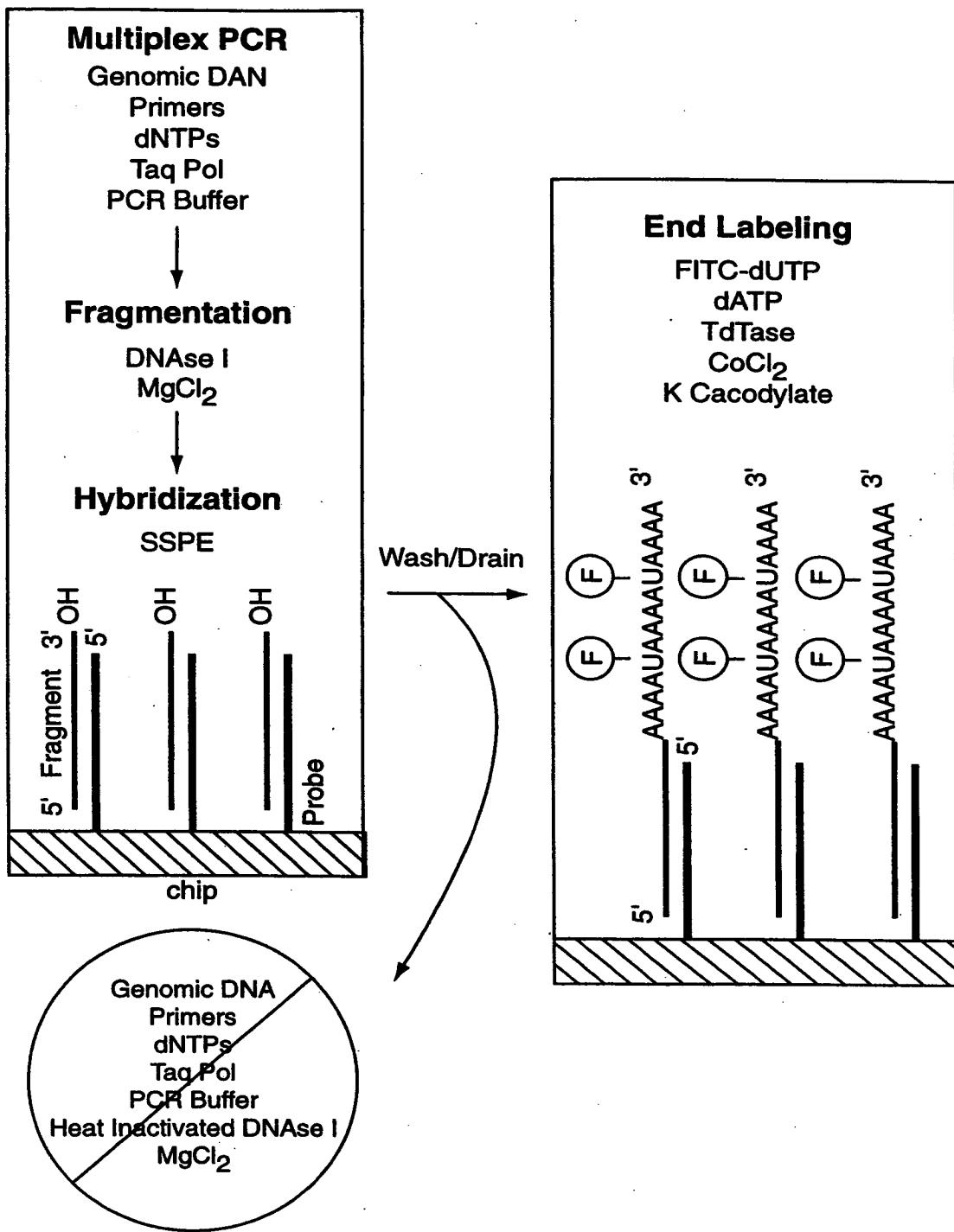


Figure 19

Pre-react Chip Prior to Hybridization and Labeling

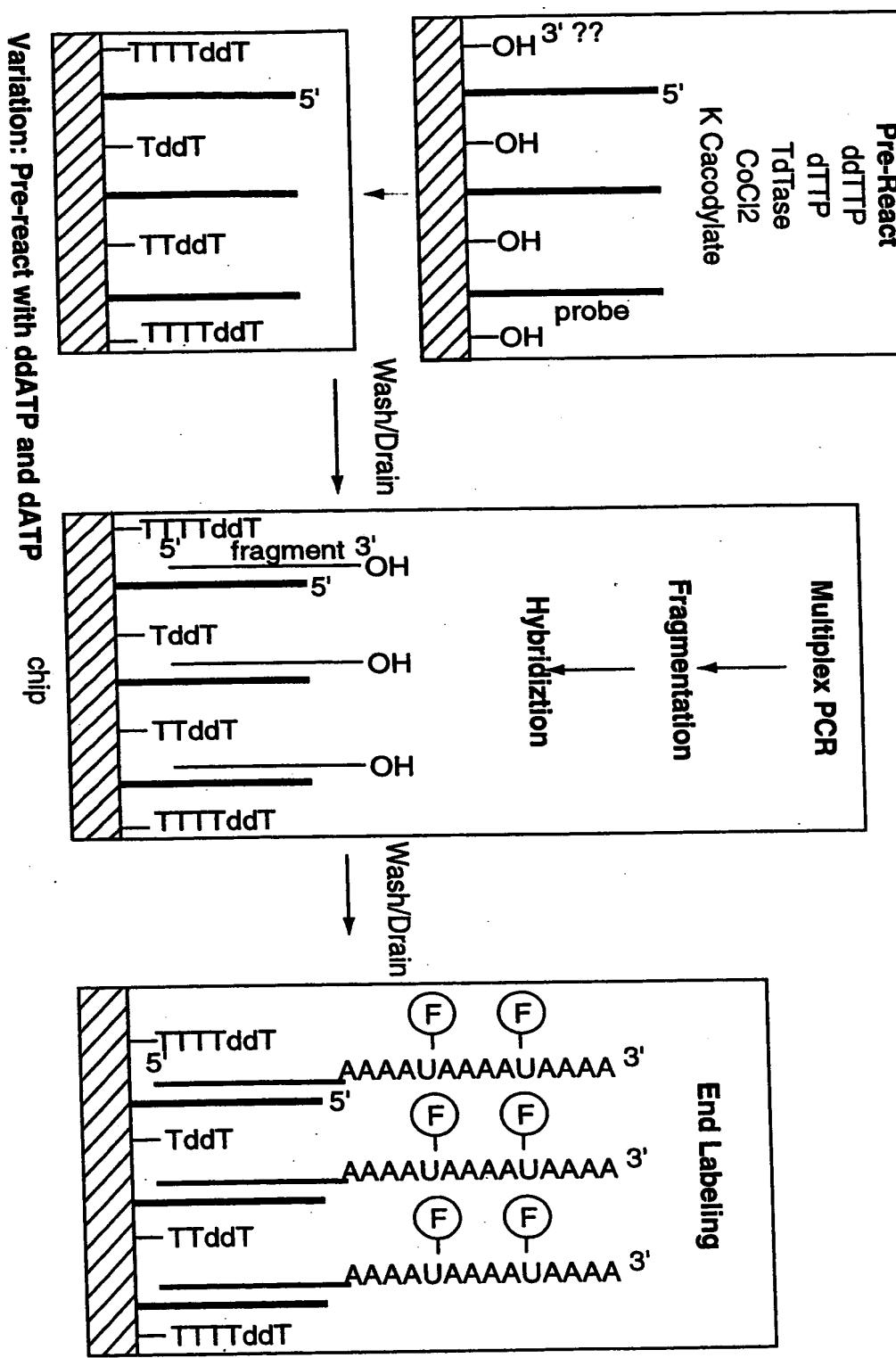


Figure 20

DNase Titration: "Ideal" Fragment Length

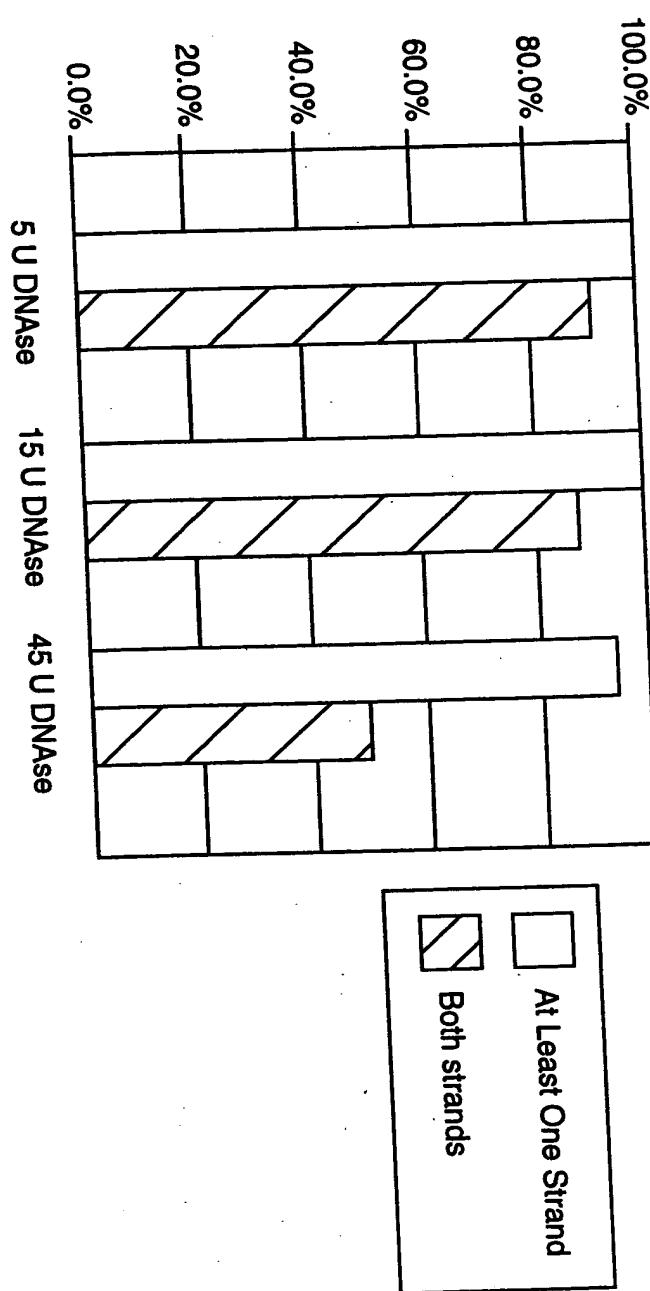
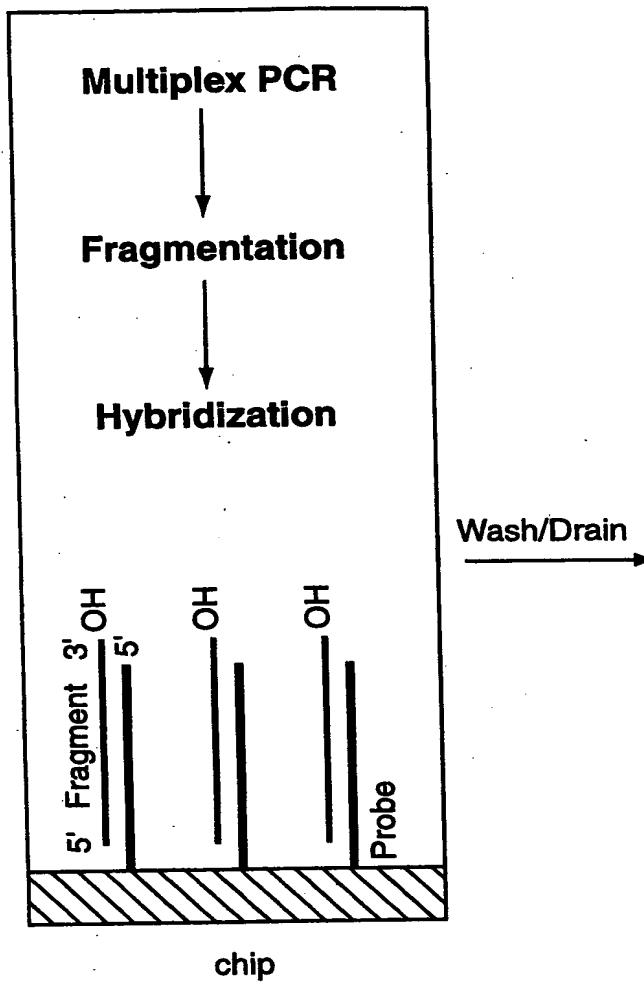


Figure 21

Oligo dT Labeling on the Chip



Substitute FITC with:
• Rhodamine R110
• Cy fluorochrome

Oligo Synthesis

**FITC-Phosphoramidite
ddT nucleoside at 3' end
dT nucleosides**

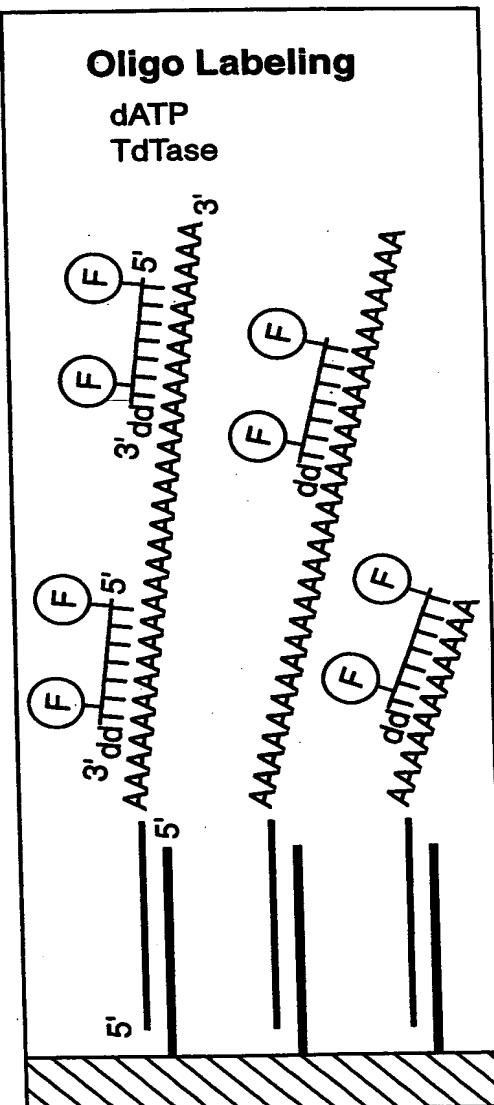
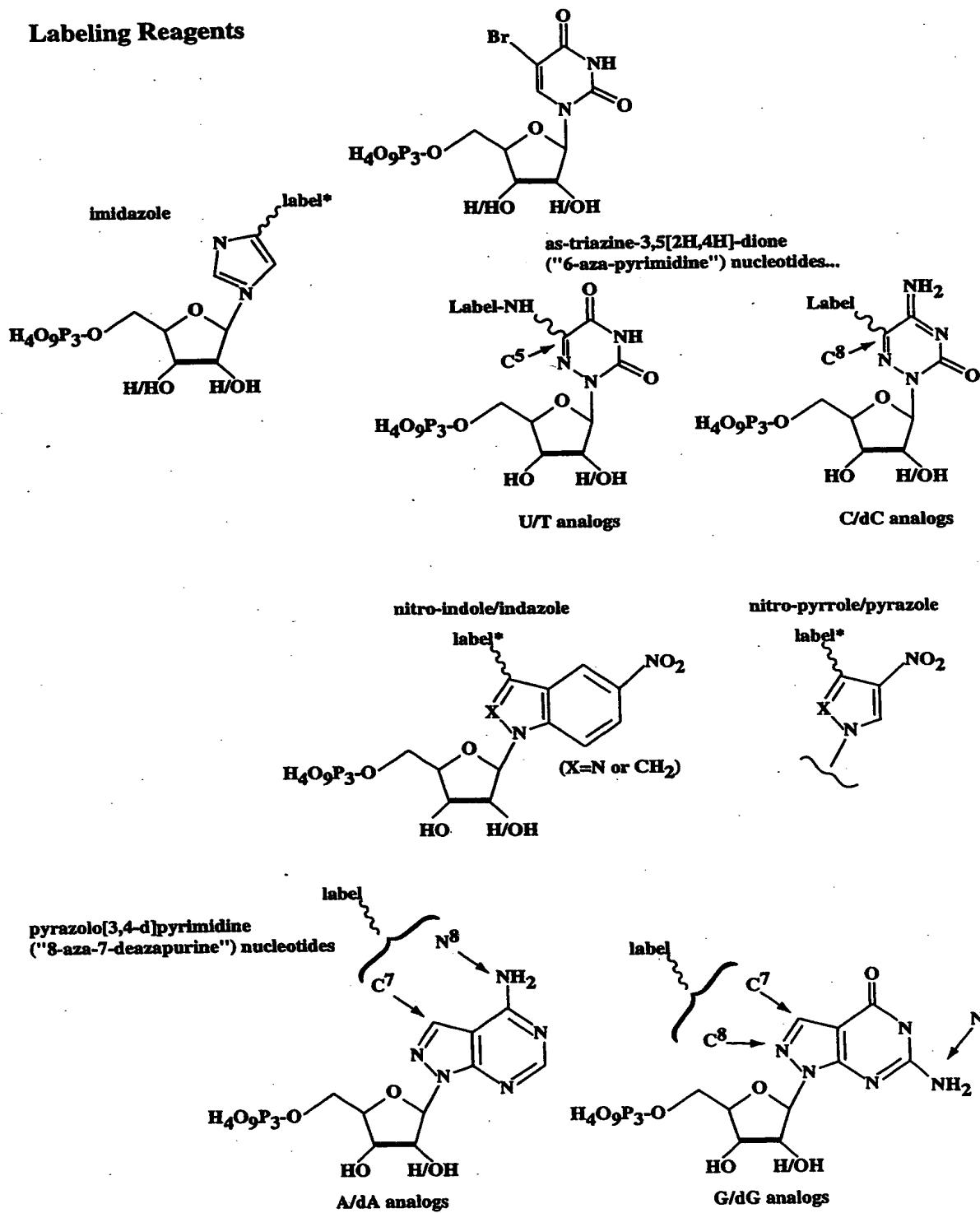


Figure 22

Labeling Reagents**Figure 23a**

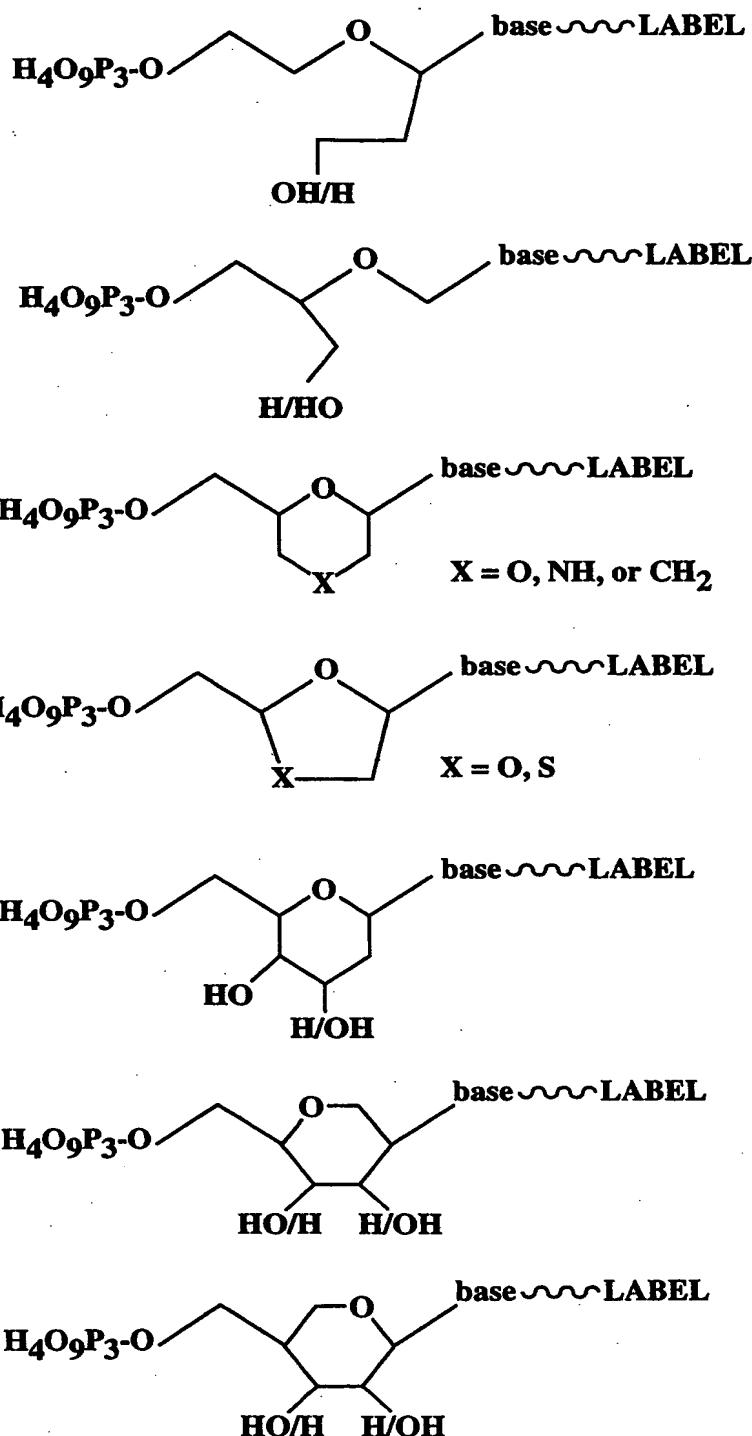
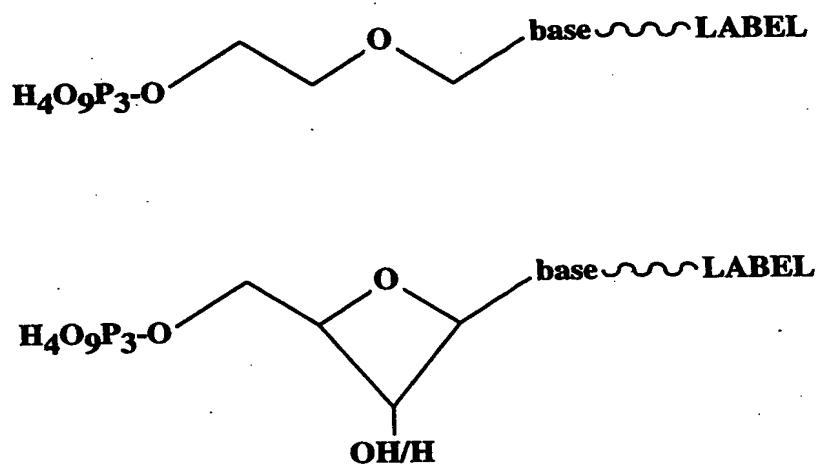
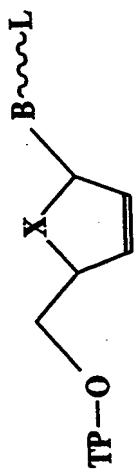
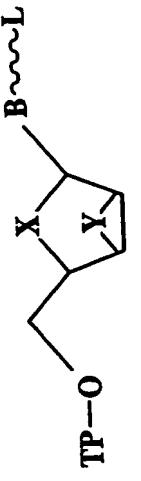


Figure 23b

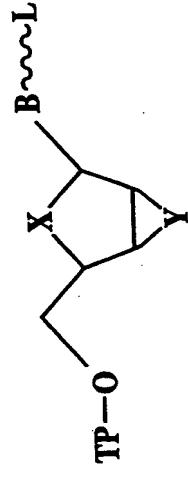


base = heterocycloc moiety (eg. analogs thereof)
~~~~ = linker;
LABEL = detectable signal-gene

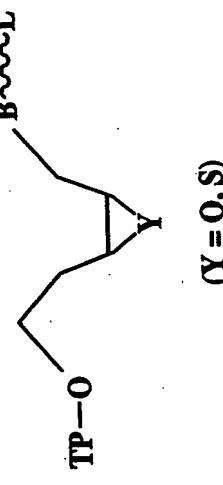
Figure 23c



(X = O, S, CH₂)



(X = O, S, CH₂)
(Y = O, S)



L = label

TP = triphosphate

Figure 23d

Resequencing a target DNA molecule with a set of generic n-mer tiling probes

i.e. 4-mer probes:

Target: 5' TGACATAGGACAGCGAAGGGA...
 3'

Probe 1: ACTG 5'

Probe 2: CTGT

Probe 3: TGTA

GTAT

Probe 5: TATC

ATCC

TCCT

CCTG

Probe 9: CTGT...etc.

Figure 24

Four electronic tiling arrays are present on a 4-mer generic array:

($4 \times 3 = 12$ "nearest neighbors" for each probe)

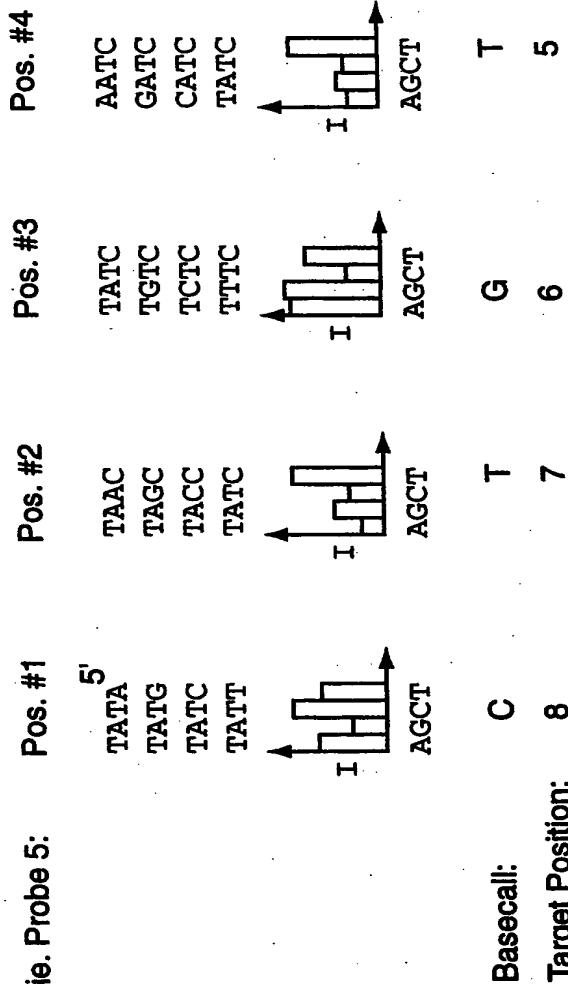


Figure 25

Base Calling at the 8th position in the target

Target: TGACATAGGACAGCGAAGGGA...
8th

Probe 5, Pos. 1	3'	5'	Base-Call
Probe 6, Pos. 2	TATC		T
Probe 7, Pos. 3	ATCC		G
Probe 8, Pos. 4	TCCT		C
	CCTG		C

C is the winner

Figure 26

Base Vote Table

Base Position	Base Identity	Vote	Score Total	Correctness Score #1	Correctness Score #2	Correctness Score #3	Correctness Score #4
5	T	T	1	1	0	1	0
6	A	A	1	1	1	1	1
7	T	T	1	0	1	0	1
9	C	G	0	0	0	0	0
10	T	T	1	1	0	1	0
11	G	G	1	0	1	1	1
12	T	T	1	0	1	1	1
13	C	C	1	1	0	0	1
14	G	G	1	0	1	1	1
(10) TOTALS							

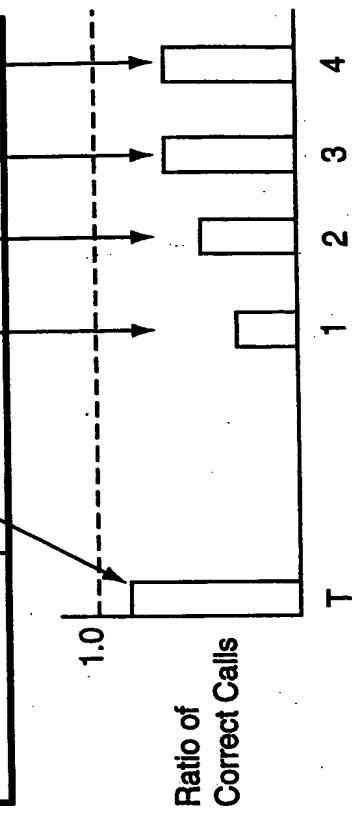
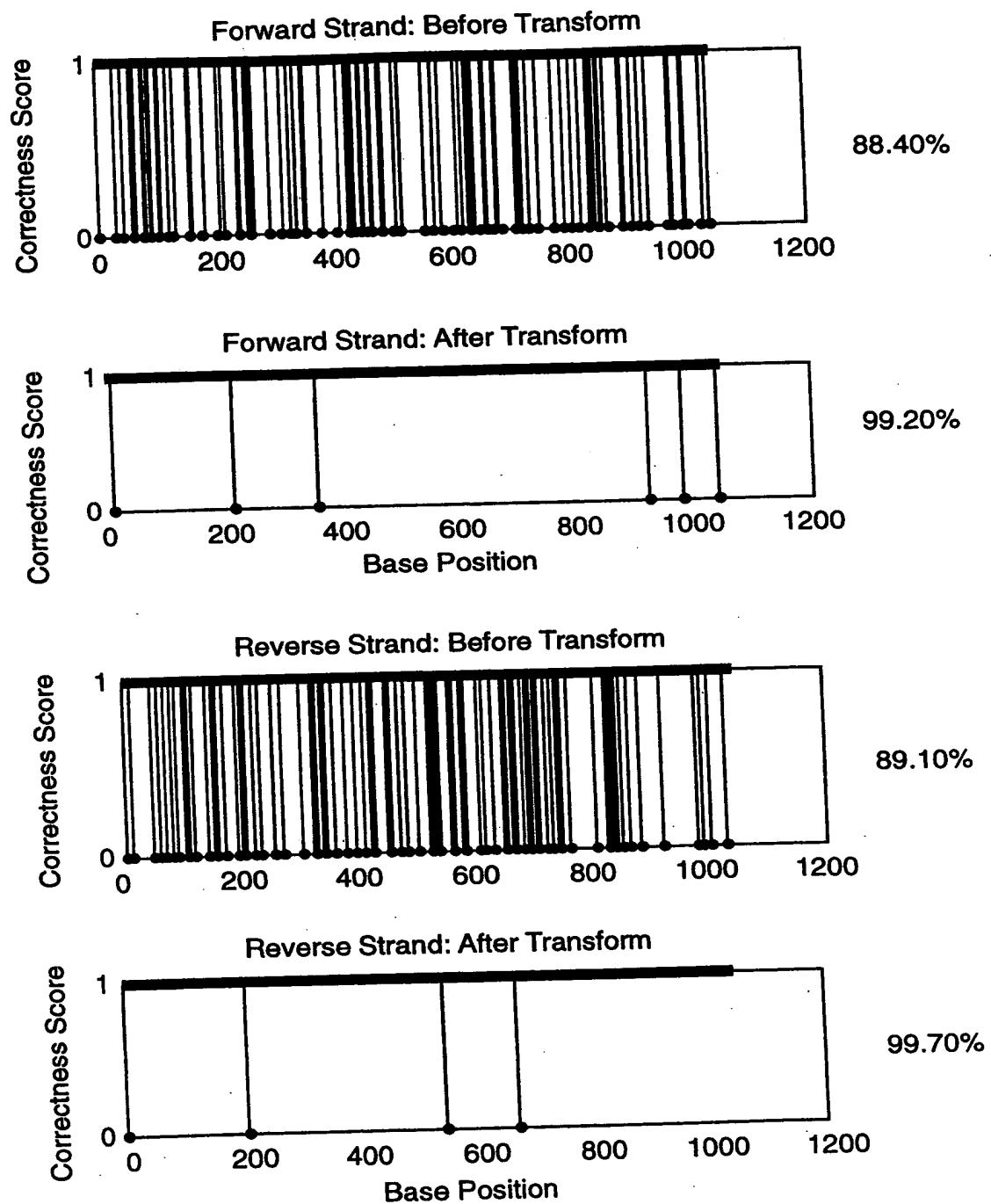
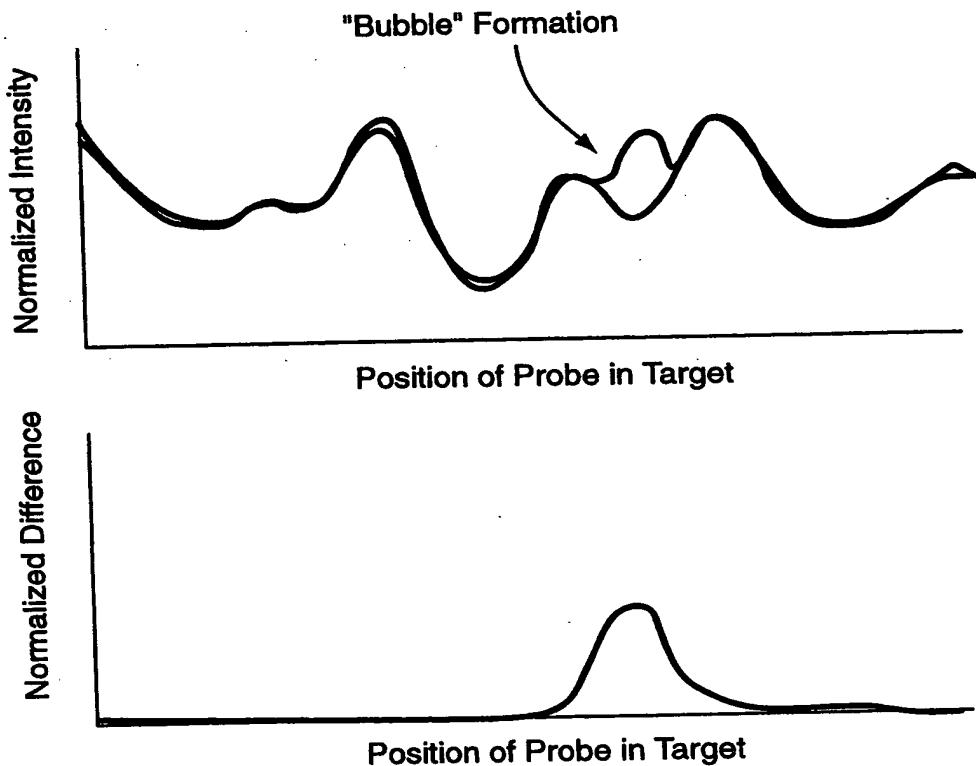


Figure 27

Effect of Applying Correctness Score Transform to HIV Data**Figure 28**

Mutation Detection by Intensity Comparisons



Algorithms:

$$I_{\text{normalized}} = I_{\text{probe}} / (\sum I_{\text{NN}})$$

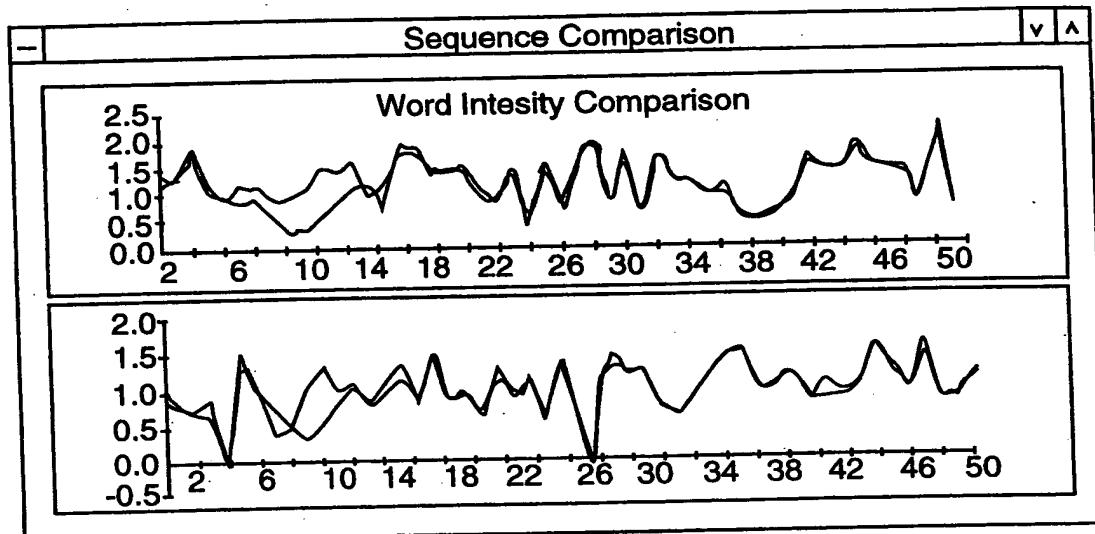
$$I_{\text{difference}} = \frac{(I_{\text{normalized, variant}} - I_{\text{normalized, control}})}{(I_{\text{normalized, variant}} + I_{\text{normalized, control}})}$$

- Locally normalized intensities track well
- Local normalization is sensitive to mutations

Figure 29

Bubble Formation Detection of Mutation in HIV Genome

Normalized Intensity Comparison:



Normalized Difference:

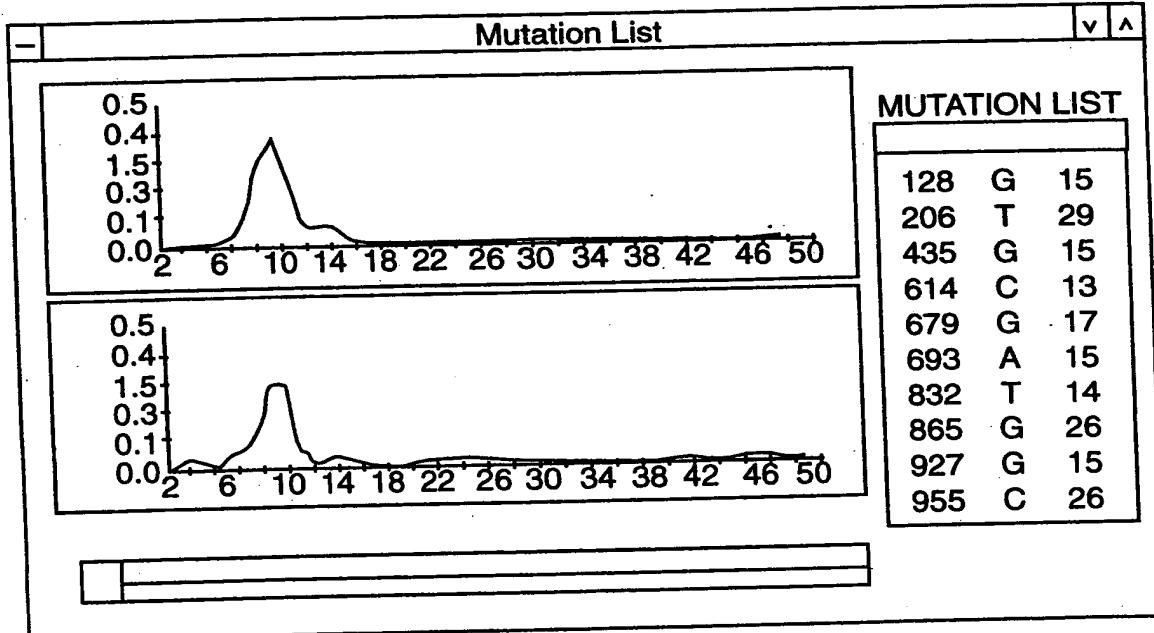
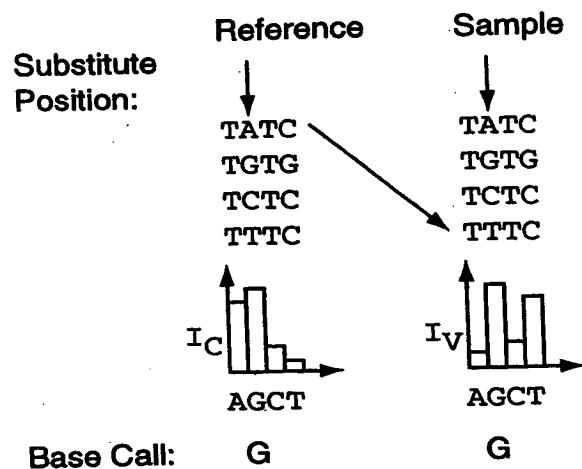


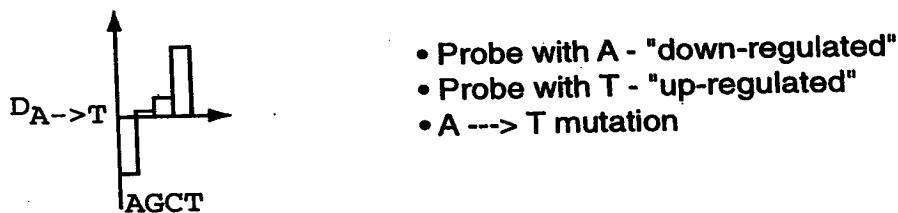
Figure 30

Induced Difference Nearest Neighbor Probe Scoring:



$$\text{Induced Difference: } D_A = (I_{V,A} - I_{C,A}) / I_{C,A}$$

- Average induced differences over all tilings and over both forward and reverse strands.

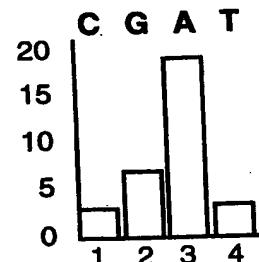


- Total Induced Difference > + Threshold: Mutation Exists
- Total Induced Difference < - Threshold: Mutation Exists
- Two criteria for mutations: Induced Difference Scores; Bubble Formation

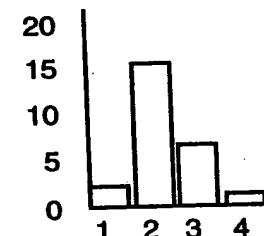
Figure 31

**Mutations found in an HIV PCR target (B) using a generic ligation
GeneChip and induced difference analysis**

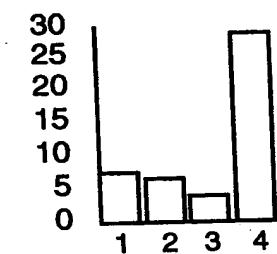
21 30 40 50
 actgtatccttagctccctcagatcact
 actgtatccttaacttccctcagatcact



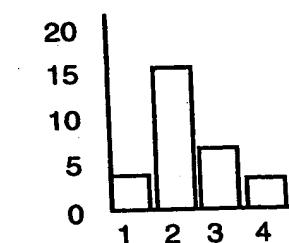
134 140 150 160
 attagaagaaatgaatttgcaggaagatg
 attagaagaaatgagttgcaggaagatg



211 220 230 240
 agtatgatcagatacccatagaaaatctgtg
 agtatgatcagatactcatagaaaatctgtg



440 420 430 440
 agaaatttgtacagaaaatggaaaaggaagg
 agaaatttgtacagrgatggaaaaggaagg



621 630 640 650
 catcccgcaagggttaaaaaagaaaaaatca
 catcccgcaagggtcmaaaaagaaaaaatca

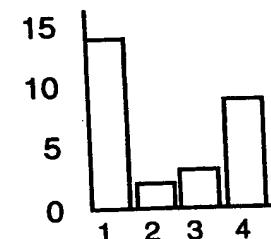
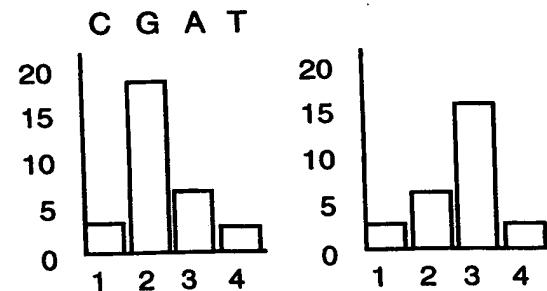
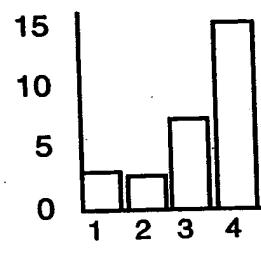


Figure 32a

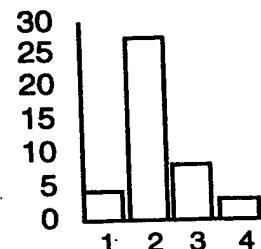
693 700 710 720
ttagataaaagacttcaggaagtatactgca
tttagatgaagacttcaggaaatatactgca



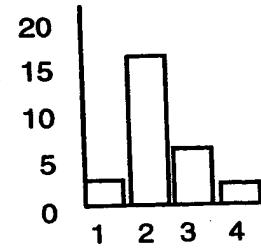
840 850 860 867
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tagagccttttagataacaaaatccagaca



872 880 890 900
tatctatcaatacatggatgatttgtatgt
tatctatcaatacgtggatgatttgtatgt



934 940 950 960
caaaaatagaggaactgagacaacatctgt
caaaaatagaggagctgagacaacatctgt



960 970 980 989
ctgtttaggtgggatttaccacaccagac
ctgtttaggtgggacttaccacaccagac

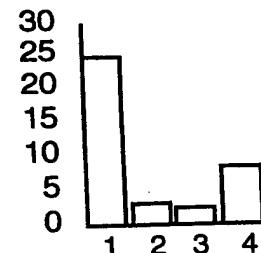
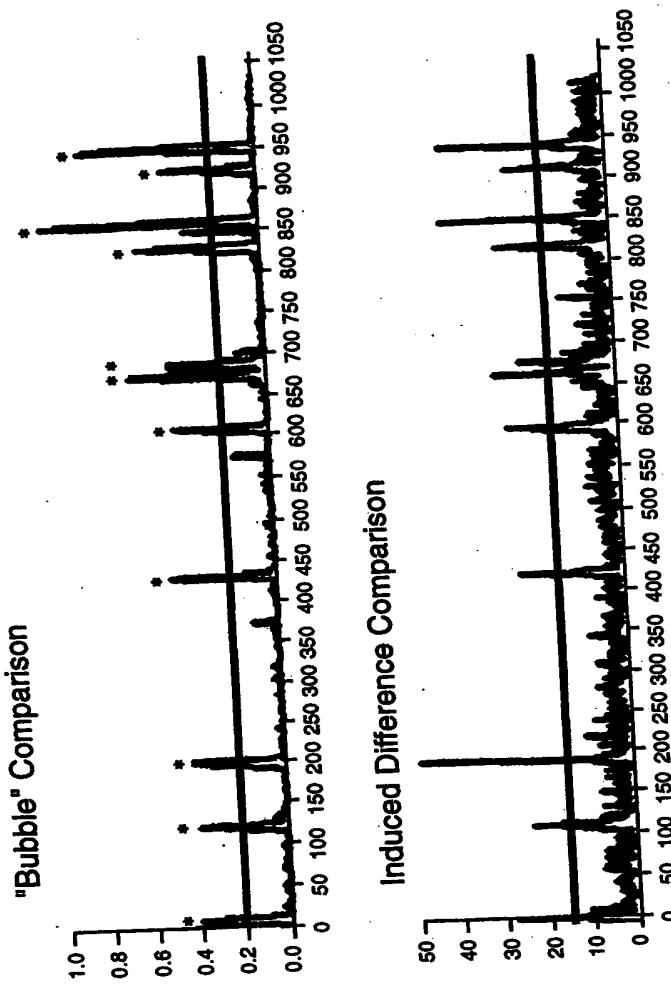


Figure 32b

47/47 47/47 47/47 47/47 47/47

Mutation Detection Using Comparisons Between a Reference Target and a Sample Target



Results: No false positives, all 11 mutations (indicated by *) are detected
in this 1041 bp HIV DNA fragment.

Figure 33